ANALYTICAL STUDIES OF PLANT GUM EXUDATES

by

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Thesis presented for the degree

of

Doctor of Philosophy

University of Edinburgh

September 1985

TO THE BRIDGEMANS

DECLARATION

I hereby declare that this Thesis was composed by myself and that the experimental studies reported are my own. None of the work included in this Thesis has been submitted for any other degree or professional qualification.

Some of the analytical data reported in Sections III, IV and VII have been published or are in press (D.M.W. Anderson, M.M.E. Bridgeman, J.G.K. Farquhar and C.G.A. McNab, Int. Tree Crops J., 1983, 2, 245; D.M.W. Anderson and M.M.E. Bridgeman, Int. Tree Crops J., 1983, 2, 291; D.M.W. Anderson, M.M.E. Bridgeman and G. De Pinto, Phytochem., 1984, 23 (3), 575; D.M.W. Anderson and M.M.E. Bridgeman, Phytochem., 1985, In press).

ACKNOWLEDGEMENTS

I would like to thank the Chemistry Department, University of Edinburgh, for the use of its laboratory facilities; Chemistry Department, University of St. Andrews, for determining nitrogen contents; Dr J. McNab, A.R.C. Poultry Research Centre, Roslin, for amino acid analyses and Mrs J. Robertson and Staff of the Animal Unit, Western General Hospital, Edinburgh for facilitating the dietary studies with rats.

My sincere thanks go to my supervisor, Dr D.M.W. Anderson for his help and encouragement throughout the period of this study.

I am indebted to the Science and Engineering Research Council and to the Ministry of Agriculture, Fisheries and Food for financial support and to Mrs E. Paterson for typing this Thesis.

ABSTRACT

The gum exudate from *Acacia calcigera*, a species recently discovered in Australia, has been shown to have a highly positive specific rotation and high molecular weight with a low rhamnose content. These results are characteristic of species within the Section *Gummiferae*, a predominantly African section of the genus *Acacia*.

Analytical data for the gum exudate from a cultivar of *Leucaena leucocephala* from India and for gum arabic (*Acacia senegal*) from Africa were compared. The *Leucaena* gum had a chemical composition and properties similar to gum arabic but was of higher viscosity and molecular weight; these differences could be commercially important if gum collection from *Leucaena* could be organised.

In a series of studies in laboratory rats, gum arabic was completely degraded on incorporation into a standard rat diet at levels of 2g/day/rat and 4g/day/rat. On incorporation into an elemental, low residue diet ('Flexical') gum arabic was partially degraded when fed to rats at 2g/day/rat but was found to be degraded more extensively if fed at a reduced level (1g/day/rat). Gum arabic, mixed with faeces from rats fed the elemental diet was partially degraded by faecal bacteria. The different results obtained when gum arabic was incorporated into two different diets indicated the importance of choice of type of diet and dose level used in dietary studies.

Faecal extracts obtained from rats fed a standard diet supplemented with gum karaya (1.2g/day/rat) were shown to be similar, but not identical, to gum karaya that had been mixed with faeces then re-extracted. A similar result was obtained when an elemental diet was used. It was not possible to conclude whether or not the gum karaya extracted from test faeces had been degraded because of the difficulties found to be associated with attempted molecular weight measurements of the impure forms of the gum extracted.

Seven commercial gum tragacanth samples from Iran were found to vary in composition and in viscosity and in the ratio of their water-insoluble and water-soluble components. Their amino acid contents did not differ extensively. Five commercial gum tragacanth samples from Turkey showed less variation than the Iranian samples; although having lower viscosity, their amino acid compositions were similar to those of the Iranian samples. A Turkish gum tragacanth sample from Astragalus microcephalus (the major source of the gum) differed extensively analytically from Turkish gum tragacanth samples from Astragalus kurdicus and Astragalus gummifer (minor sources).

The Test Article used in a dietary study of gum tragacanth in Man was shown to have been well-chosen, representing gum tragacanth of fair average quality.

The water-insoluble and water-soluble components of the gum tragacanth samples studies were found to have similar sugar and ami-

no acid compositions, although the water-insoluble components had higher protein contents and lower ash and methoxyl contents than the water-soluble components.

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SECTION I

GENERAL INTRODUCTION

Many plant families include species that exude gums to a greater or lesser degree. Gums may be exuded only in very small amounts and may not be readily discernible or they may be produced copiously forming large conspicuous incrustations.

Plants that produce gum are usually shrubs or low growing trees from which the gum exudates as vermiform or tear-shapes and may build up in thickened layers. Harvesting is by hand-picking, usually by native workers in countries where the labour costs are low (1).

The term 'gum' designates a great variety of natural products in the form of tears, flakes and angular fragments, sticky in nature, found on the stem surfaces of trees. However, true gums may be regarded as the exudation products of certain trees in the form of complex polysaccharides. They are dispersible in hot or cold water but insoluble in alcohol or other organic solvents (2).

Gums come from a variety of plants and today, usage of the natural water-soluble gums is growing at a rate of 10% per annum. But economics is changing the types of gums that are used. Labour cost escalation, even in remote areas, and the quality control needed for relatively pure and constant quality material cause some plant sources to be eliminated and others, perhaps more amenable to cultivation, to appear as new sources (1).

Gums are carbohydrate polymers composed of sugar units

glycosidically condensed to form large molecules. As a consequence, they are, for the most part of high molecular weight, some linear and some branched (1).

Gums are also proteinaceous. Protein contents can vary from close to zero e.g. in Sterculia setigera gum (3) to between 30% and 40% e.g. in Neem gum (Azadirachta indica) (4) and, more recently, to as much as 53% e.g. in Acacia difficilis gum (5).

Gums are widely used in medicines, foods, cosmetics, adhesives, paints, inks, textiles etc (2). They are excellent suspending agents, dispersants, stabilising agents, emulsifying and gel-forming agents. They are also used as coagulants, binders, lubricants and film formers.

The main use of plant gum exudates is in the food industry where they are used as food additives. It is now rare for a processed food not to contain a gum product (i) to correct or minimise defects in its natural ingredients, (ii) to increase the sensory satisfaction derived and (iii) to produce formulations that make possible new combinations of food ingredients (6,7).

Generally, food additives are used as preservatives, antioxidants, emulsifiers, thickeners, stabilisers, artificial flavourings, maturing and bleaching agents, non-nutritive sweeteners or to produce nutritive value (e.g. added vitamins).

World-wide population growth and improved standards of living, call for an increased food supply. This cannot only result from increased food production but also from better protection and preservation of food supplies and the use of better processing techniques in line with advances in food technology. The use of such techniques implies an increased use of food additives (8). The progress of industrialisation requires studies regarding the toxicity of food additives which have traditionally been regarded as safe.

In the analysis of plant gum exudates, a number of analytical parameters can be used to express the chemical composition and physical properties of a gum. The parameters used to characterise gums are ash, protein and methoxyl contents, specific optical rotation, intrinsic viscosity, molecular weight, equivalent weight and the ratios of sugars present after acidic hydrolysis. The analytical parameters taken overall establish a form of 'fingerprint' which serves to characterise each species of gum.

Section III of this Thesis presents analytical data for a gum exudate from Acacia calcigera a species only recently discovered in Australia.

Section IV presents analytical data for a gum exudate from Leucaena leucocephala from South India; the data are compared with those for gum arabic (Acacia senegal).

Section V and Section VI respectively, concern the use of

analytical techniques to try to establish the extent of degradation (if any) of gum arabic and gum karaya after ingestion by the rat.

Section VII presents analytical data for several commercial gum tragacanth (Astragalus spp.) samples of Iranian and Turkish origin and samples collected from named Turkish species. These studies were required for regulatory purposes concerning the tests for identity and purity of gum tragacanth and to try and explain the contradictory statements in the literature and to characterise a sample of gum used as the Test Article in a dietary study in Man.

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SECTION II

METHODS USED IN THE ANALYTICAL STUDY OF PLANT GUM EXUDATES

II.1 GENERAL METHODS

Weighings. All accurate weighings were made using a Stanton Unimatic Model C.L.I. single-pan balance having an accuracy of ±0.1mg.

Dialyses of polysaccharides, to remove low molecular weight material, were carried out in Visking cellophane tubing (Kalle Aktiengesellschaft, Wiesbaden) against running tap water for 48-72 hours and then against distilled water for 48 hours.

Electrodialyses of polysaccharides were carried out in a three compartment perspex cell fitted with cellophane membranes. The water in the outer electrode compartments was changed regularly to prevent overheating. Electrodialysis was continued until a current (applied voltage = 300 volts) ceased to flow.

Reductions in volume were carried out on a rotary evaporator at temperatures below 40°C unless otherwise stated.

Moisture contents were determined by heating to constant weight at 105°C.

Ash contents were determined by heating to constant weight in a muffle furnace at 550°C.

Carbon, hydrogen and nitrogen contents were determined with a Perkin-Elmer 240 Elemental Analyser.

Methoxyl contents were determined by a vapour phase infra red method [1,2]; a calibration curve was based on known weights of methyl iodide. Infra red spectroscopy was carried out using a Perkin-Elmer 137 spectrophotometer.

Equivalent weight determinations on exhaustively electrodialysed polysaccarides were carried out by direct titration with standard sodium hydroxide solution (ca. 0.01N).

Uronic acid contents were calculated from the equivalent weights as 17600/E.W. i.e. values are expressed as uronic anhydride.

II.2 PHYSICAL METHODS

Specific rotations of aqueous solutions were measured using the sodium D-line with a Perkin-Elmer model 141 polarimeter at $20\pm2^{o}C$. All solutions were first clarified by passage through filters of average pore size 0.8μm (Millipore Ltd., Bedford, Mass., USA) with a stainless steel filter holder attached to a syringe (20ml). Concentrations of gum were assumed to be unaltered by ultrafiltration [3].

Intrinsic viscosity determinations were carried out in M-sodium chloride solution in an Ubbelohde suspended-level dilution

viscometer at 25.0±0.1°C. A sample of gum (100-200 mg) was dissolved in M-sodium chloride solution (10 ml). The solution was filtered as for specific rotation analysis before addition to the viscometer. Flow times were measured to within 0.1 second by means of a stopwatch. The isoionic dilution method was used; the gum solution (6ml) was placed in the viscometer and the flow time was measured. Flow times were also obtained for successive dilutions with M-sodium chloride solutions (four additions each of 2ml). Since preliminary experiments had indicated that any loss of gum from M-sodium chloride solution on filtering was negligible, concentration values were estimated from the dry weight of the gum dissolved in a known volume.

Assuming the densities of M-sodium chloride and the gum solutions to be equal for low concentrations of gum, the limiting viscosity number $[\eta]$ is given by:

$$[\eta] = \lim_{c \to 0} \eta_{sp}/c = \lim_{c \to 0} (t - t_o)/ct_o$$

where c is the concentration of the gum solution (g/ml) and t_o and t are the flow times (seconds) for solvent and solution respectively.

Extrapolation of the linear plot of $(t - t_o)/ct_o$ vs c to c = 0 gives $[\eta]$ the limiting viscosity number (alternatively known as the intrinsic viscosity)(9).

Light scattering measurements, for molecular weight determinations were carried out at 28.0±0.5°C with a SOFICA photogoniodiffusometer Model 4200. Unpolarised green light (546 nm) was selected from a mercury lamp spectrum with a Wratten Kodak N61 filter. Using the limiting viscosity number as a guideline to the desirable concentration and using M-sodium chloride as a solvent, gum solutions were accurately prepared (0.1 - 0.3g in 50 ml). Dilutions of this solution were made and the molecular weight was calculated as an average of three of these solutions. The solutions were clarified and made dust free by passage through filters of average pore size 0.8μm and 0.22μm (Millipore Ltd., Bedford, Mass., USA) respectively, using a stainless steel holder attached to a syringe (20ml).

For each concentration, the intensity of scattered light at various angles between 30° and 150° was recorded and corrected, and corrected scale readings I_θ for angle θ were calculated [4] from the equation:

$$I_\theta = \frac{(I_{soln} - I_{sol})}{1 + \cos^2\theta} \operatorname{Sin}\theta$$

where I_{soln} and I_{sol} are the scale readings for the polymer solution and solvent respectively. The reciprocal corrected scale reading $1/I_\theta$ is plotted against $\operatorname{Sin}^2\theta/2$. Extrapolation of the linear portion of this graph to θ = 0 gives a value for $[1/I_\theta]_{\theta=0}$. The downward curvature of these graphs at low angles is thought to be caused by dust particles suspended in solution [5].

Molecular weights are then calculated from the equation:

$$M = \frac{R}{\frac{2\pi^2 n_o^2}{\lambda^4 N} \cdot [dn/dc]^2 \cdot I_B \cdot c \cdot [1/I_\theta]_{\theta=0}}$$

where n_o = refractive index of solvent (1.340)

n = refractive index of solution

N = Avogadro's number (6.023×10^{23})

λ = wavelength of incident light (546 nm)

c = concentration in g/ml

I_B = intensity diffused, selected for standard benzene (0.5)

dn/dc = refractive index increment

R = Rayleigh constant of standard benzene (16.3×10^{-6} for λ = 546 nm)

Using the dn/dc value of 0.146 (i.e. the average value found [6] of a series of Acacia gums) the equation is reduced to:

$$M = 2.309 \times 10^2/(c.[1/I_\theta]_{\theta=0}).$$

II.3 CHEMICAL METHODS

(i) Small scale polysaccharide hydrolyses were carried out overnight with 0.5M - 1.0M sulphuric acid in a boiling water bath. Hydrolysates were neutralised with barium carbonate, vacuum filtered, deionised with Amberlite IR-120 (H) resin, vacuum filtered and concentrated to a syrup on a rotary evaporator.

(ii) Chromatographic separations

Paper chromatography of sugars was carried out on Whatman No. 1 paper (unless otherwise stated) using the following solvent systems (v/v):

(a) ethyl acetate, acetic acid, formic acid and water (18:3:1:4),

(b) benzene, butanol, pyridine and water (1:5:3:3, upper layer)

(c) ethanol, hydrochloric acid (0.1N) and butanol (10:5:1)[7].

Before using solvent system (c), the papers were dipped in 0.3M sodium dihydrogen ortho phosphate solution and air dried.

Reducing sugars were detected by spraying chromatograms with a saturated solution of aniline oxalate in ethanol/water (1:1, v/v), followed by heating at ca. 150^{o}C for ca. 5 minutes.

(iii) Quantitative determination of sugars

Sugars were separated from hydrolysates by chromatography in solvent (b) or (c) on Whatman 3MM papers. After elution from the papers, sugars were quantified by the phenol-sulphuric acid method [8]. The absorbances were read using a Unicam SP1300 spectrophotometer with filter 2. Calibration curves were obtained from known

weights of sugars.

(iv) Amino acid hydrolysis

Sufficiently finely ground sample to give nitrogen (2mg) (crude protein, 12.5mg) was weighed and transferred quantitatively to a round-bottomed two-necked flask (100ml). After adding anti-bumping granules, 6N hydrochloric acid (Analar, specific gravity 1.18:H_2O 1:1) (80ml) and 0.004M nor-leucine (0.5248g/litre in N/10 hydrochloric acid)(2ml), the flask was fitted to an air cooled condenser (800mm) and the apparatus purged with oxygen-free nitrogen gas. The contents of the flask were heated under reflux for exactly 20 hours under a continuous and slow stream of oxygen-free nitrogen gas. The flask was allowed to cool and the condenser was washed with water. The solution was vacuum filtered using No. 42 Whatman filter papers and evaporated to dryness at 42°C. The residue was dissolved in N/100 hydrochloric acid (20ml), filtered through a filter of average pore size 0.22µm (Millipore Ltd., Bedford, Mass., USA) with a stainless steel filter holder attached to a syringe (20ml), and stored in a glass vial pending analysis.

(v) Amino acid analysis

A suitable aliquot (normally 50µl) was applied to a stainless steel column (350mm x 3mm) of cationic exchange resin (6µ beads from Rank Hilger) and the constituent amino acids separated by high pressure (ca. 2000lbs/in^2) elution with lithium citrate buffers of

increasing ionic strengths and pH. The eluted amino acids were detected by reaction with ninhydrin in a continuous flow analytical system and quantified by reference to standard solutions at 570nm (440nm for proline and hydroxyproline).

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SECTION III

AN ANALYTICAL STUDY OF THE GUM EXUDATE

FROM *ACACIA CALCIGERA*

III.1 INTRODUCTION

The genus Acacia (Family Leguminosae, sub-family Mimosoideae) is extremely large providing many complex problems of nomenclature and classification. The number of species in this genus is not known with certainty; earlier estimations varied from 500 - 900 [1,2,3], but it is now clear that the number probably exceeds 900 [4].

The genus was divided into six series by Bentham [5] although revisions have been necessary with the discovery of new species, Bentham's main divisions based on habit, inflorescence and geographical distribution are still used and are outlined below.

SERIES	NAME
1	PHYLLODINEAE
2	BOTRYOCEPHALAE
3	PULCHELLAE
4	GUMMIFERAE
5	VULGARES
6	FILICINAE

Species from Series 1 are native to Australia, Hawaii and New Caledonia; species from Series 2 and 3 are native to Australia; species from series 4 and 5 are found throughout tropical and semi-tropical parts of the world and species from series 6 are native to

South America.

Comparisons of the analytical and structural data for ca. 100 species belonging to Bentham's, series 1, 2, 4 and 5 have been made; in general, the chemical evidence substantiates [6] Bentham's taxonomic divisions. Gum exudates from Bentham's series 3 and 6 have not yet been studied.

The Acacia gum exudate studied in this section is from Acacia calcigera, an Australian species discovered recently by Dr Mary Tindale of the Royal Botanic Gardens, Sydney, N.S.W., and assigned on the basis of its botanical characteristics to Bentham's Series Gummiferae, members of which are not normally found in Australia.

Each Acacia species exudes a gum that is characteristic of that species regardless of where it is grown geographically. The aim of this section was to make an analytical comparison between the gum exudate from Acacia calcigera and the analytical parameters established for other members of the Gummiferae Series in order to ascertain if the analytical data support the assignment of this species to the Series Gummiferae.

III.2. ORIGIN OF THE GUM SPECIMEN

The gum exudate from Acacia calcigera Tindale was collected by Dr Mary Tindale and C. Dunlop on 9th July 1979, 28.8 km South of the Maranboy turnoff on Stuart Highway, Northern Territory, Aus-

tralia. The botanical specimen voucher for this tree is NSW 108559.

III.3 ANALYTICAL RESULTS AND DISCUSSION

Before analysis the nodules of gum were crushed to a fine powder using a mortar and pestle. Analytical studies were carried out on the crude gum.

The analytical results for the gum exudate from Acacia calcigera are shown in Table III.1 and compared in Table III.2 with the analytical data for five other members of the Gummiferae Series.

The Gummiferae Series is a predominantly African group of Acacias and it is unusual for a species within this Series to occur in Australia. The gum exudates from Acacia calcigera, a species recently discovered in Australia by Dr Mary Tindale, has been shown to exhibit a highly positive specific rotation and high molecular weight, and to contain low amounts of rhamnose. These results are characteristic of members of the Gummiferae Series.

The data for the gums from typical members of the Gummiferae Series e.g. Acacia drepanolobium [7], Acacia nilotica [8], Acacia nubica [9], Acacia seyal [10] and Acacia arabica [11], shown in Table III.2, correspond closely to the data obtained for the gum from Acacia calcigera e.g. nitrogen, methoxyl, galactose, arabinose and rhamnose contents, specific rotations, intrinsic viscosities and

molecular weights.

Hence the gum exudate from Acacia calcigera, despite its geographical origin, can be regarded as a typical member of Bentham's Gummiferae Series. This independent analytical evidence of the correctness of the botanical assignment of Acacia calcigera, made on the basis of the external morphological characters of the tree, was welcomed by Dr Mary Tindale.

TABLE III.1 : Analytical data for the gum exudate from *Acacia calciqera* Tindale

Moisture, %	13.1
Ash, %[a]	2.7
Nitrogen, %[a]	0.15
Hence protein, %[a]	0.90
Methoxyl, %[b]	0.76
Specific rotation, degrees[b]	+97
Intrinsic viscosity $ml g^{-1}$[a]	15
Molecular weight, Mw x 10^6[a]	2.6
Equivalent weight,[b]	1430
Hence uronic anhydride, %[b,c]	12
Sugar composition after hydrolysis:-	
4-O-Methyl glucuronic acid[d]	4.5
Glucuronic acid	7.5
Galactose	34
Arabinose	54
Rhamnose	<1

Footnotes

a : corrected for moisture content

b : corrected for moisture and protein content

c : if all acidity arising from uronic acid groups

d : if all methoxyl content present in this acid

nd : not determined

TABLE III.2 : Comparison of the analytical data for some Gummiferae species of Acacia with those found for Acacia calcigera

	Acacia drepano-lobium (7)	Acacia nilotica (8)	Acacia nubica (9)	Acacia seyal (10)	Acacia arabica (11)	Acacia calcigera
Ash, %[a]	2.52	2.48	1.54	2.87	nd	2.7
Nitrogen, %[a]	1.11	0.02	0.20	0.14	0.07	0.15
Methoxyl, %[b]	0.43	0.96	0.05	0.94	0.88	0.76
Specific rotation degrees[b]	+78	+108	+98	+51	+112	+97
Intrinsic viscosity, $ml g^{-1}$[a]	17.8	9.5	9.8	12.1	9.9	15
Molecular weight, $Mw \times 10^6$[a]	0.95	2.2	0.87	0.85	2.3	2.6
Equivalent weight[b]	1980	1890	3030	1470	1880	1430
Hence uronic anhydride, %[b,c]	9	9	7	12	10	12
Sugar composition after hydrolysis;						
4-O-Methyl glucuronic acid[d]	2.5	6	0.5	5.5	6	4.5
Glucuronic acid	6.5	3	6.5	6.5	4	7.5
Galactose	38	44	33	38	32	34
Arabinose	52	46	59	46	57	54
Rhamnose	1	0.4	1	4	0.4	<1

For a, b, c, d and nd see Table III.1.

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SECTION IV

AN ANALYTICAL STUDY OF THE GUM EXUDATE

FROM AN INDIAN CULTIVAR OF *LEUCAENA LEUCOCEPHALA*

IV.I INTRODUCTION

The tree Leucaena leucocephala (Lam) de Wit (family Leguminosae , sub-family Mimosoideae) is native to Central America but has been introduced to the Pacific Islands, the Philipines, Indonesia, Papua New Guinea, Malaysia and East and West Africa.

Leucaena leucocephala, of all tropical legumes offers a wide variety of uses to both animals and human beings. It provides nutritious forage in the form of proteinaceous fodder for animals and edible beans for humans.

Leucaena trees grow rapidly and yield an extraordinary amount of wood hence they are an abundant source of firewood and rich organic fertiliser. Their rapid growth encourages their use in revegetating tropical hillsides and in providing windbreaks, firebreaks, shade and ornamentation. A further advantage of Leucaena leucocephala is its high resistance to pests and diseases.

There are however disadvantages involved. There is a variety of Leucaena, particularly of the Hawaiian type, which has become a weed, creating problems where the plant is not desired or not harvested regularly.

The leaves of Leucaena contain an uncommon amino acid, mimosine, which is toxic to non-ruminants as it causes a reduction in the production of the thyroid hormone thyroxine. This leads to loss

of rump and tail hair and can result in goitre i.e. swollen thyroid glands. Ruminants have stomach micro-organisms that can convert mimosine into dihydroxy pyridine (DHP) which will only cause problems if ruminants consume Leucaena leaves in excessive amounts for a long period of time (3).

In recent years the many attractive ecological properties of Leucaena leucocephala have stimulated widespread interest in various aspects of its cultivation. It shows great genetic diversity; there are at least 100 different variants, based on 3 main types ascribed to Hawaii, Salvador and Peru. Such diversity offers the plant breeder opportunities for eliminating undesirable properties of Leucaena and for exploiting others [1,2,3,4].

In this section a sample of the gum exudate from a cultivar of Leucaena leucocephala was analysed in order to determine whether it possesses any properties of commercial value or interesting chemical features.

IV.2 ORIGIN OF GUM SAMPLE

The sample of gum was received from Mr. R.M. Eggenberger, Matramandir Peace Gardens, at Auroville, near Pondicherry in South India. The tree involved (age 2.5 years) produced gum copiously and did not appear to be diseased in any way. However, none of the other cultivars at Auroville yielded gum.

IV.3 ANALYTICAL RESULTS AND DISCUSSION

The analytical results for the gum exudate from Leucaena leucocephala are shown in Table IV together with the corresponding data for gum arabic [5,6] derived from Acacia senegal (L). Willd. The amino acid profiles for both gum samples are shown in Figure IV.1.

Leucaena leucocephala belongs to the family Leguminosae, sub-family Mimosoideae and therefore can be expected to have a close relationship to other well-known gum bearing genera of the same family and sub-family e.g. Acacia, Prosopis.

It was therefore of interest to determine whether the Leucaena gum exudate bore any close chemical or physico-chemical properties to any of the gum exudates from species in Mimosoideae studied previously.

The genus Acacia (family Leguminosae , sub-family Mimosoideae) was divided into six sections by Bentham [7] as detailed in Section III. The principal source of gum arabic, an extremely important commercial gum, is Acacia senegal which is placed in Benthams section V (Vulgares).

The gum exudate from Leucaena leucocephala was found to have a negative optical rotation and a low nitrogen content with a ratio of glucuronic acid to rhamnose close to unity. The three principal amino acids in the Leucaena gum exudate and in gum arabic (Acacia

senegal) were hydroxyproline, serine and proline. However, the *Leucaena* gum exudate had increased amounts of alanine, arginine, isoleucine, lysine, serine and tyrosine when compared to gum arabic (*Acacia senegal*). The chemical composition and properties of *Leucaena* gum bore a close resemblance to those for gum arabic, as can be seen in Table IV and Figure IV.1. *Leucaena* gum is, however more viscous and of a higher molecular weight than gum arabic; these advantages are commercially important.

If a gum yielding cultivar of *Leucaena leucocephala* could be utilised in some of the future planting projects which are ecologically desirable, particularly in arid zones, then the scale of planting involved in such operations could lead to the production of considerable amounts of *Leucaena leucocephala* gum which would undoubtedly be of commercial interest for applications in which gum arabic is traditionally used.

TABLE IV : Analytical data for the gum exudate from Leucaena leucocephala

Moisture	17.7	(13.1)
Ash, %[a]	4.4	(3.8)
Nitrogen, %[a]	0.37	(0.37)
Hence protein, %(N x 6.25)[a]	2.3	(2.3)
Methoxyl, %[b]	0.92	(0.25)
Specific rotation, degrees[b]	-28	(-30)
Intrinsic viscosity, ml g^{-1}[a]	24.1	(13.4)
Molecular weight, $\bar{M}w \times 10^6$[a]	1.7	(0.58)
Equivalent weight,[b]	767	(1085)
Hence uronic anhydride, %[b,c]	23	(16)
Sugar composition after hydrolysis:		
Glucuronic acid	17.5	(16.0)
4-O-Methyl glucuronic acid[d]	5.5	(1.5)
Galactose	36	(40)
Arabinose	22	(28)
Rhamnose	19	(14)

* ( ) corresponding data for gum arabic (5,6)

Footnotes

a : corrected for moisture content

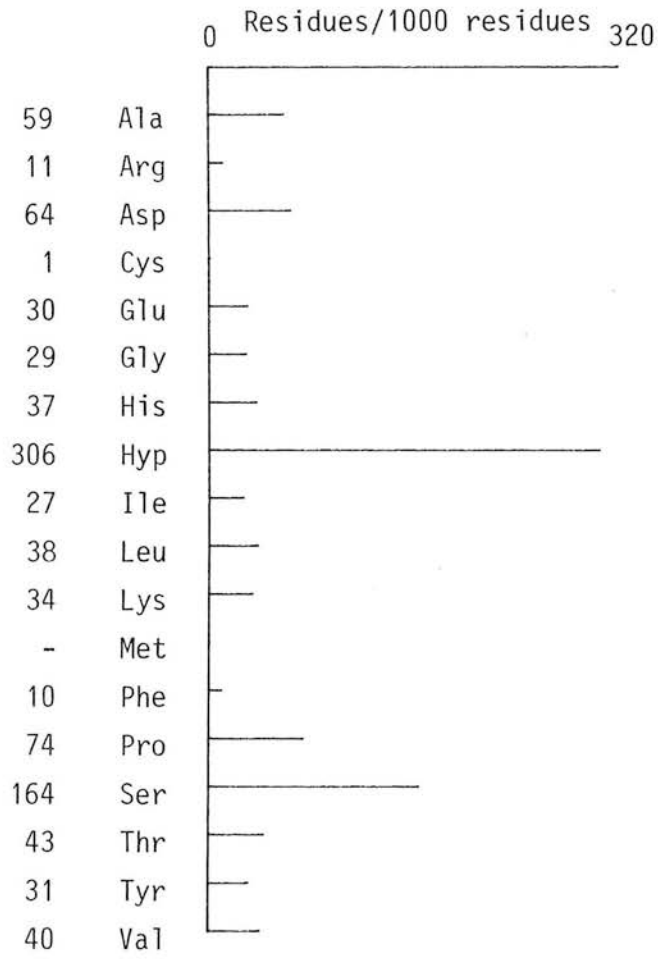
b : corrected for moisture and protein content

c : if all acidity arising from uronic acid groups

d : if all methoxyl content present in this acid

FIGURE IV.1 : Amino acid profiles

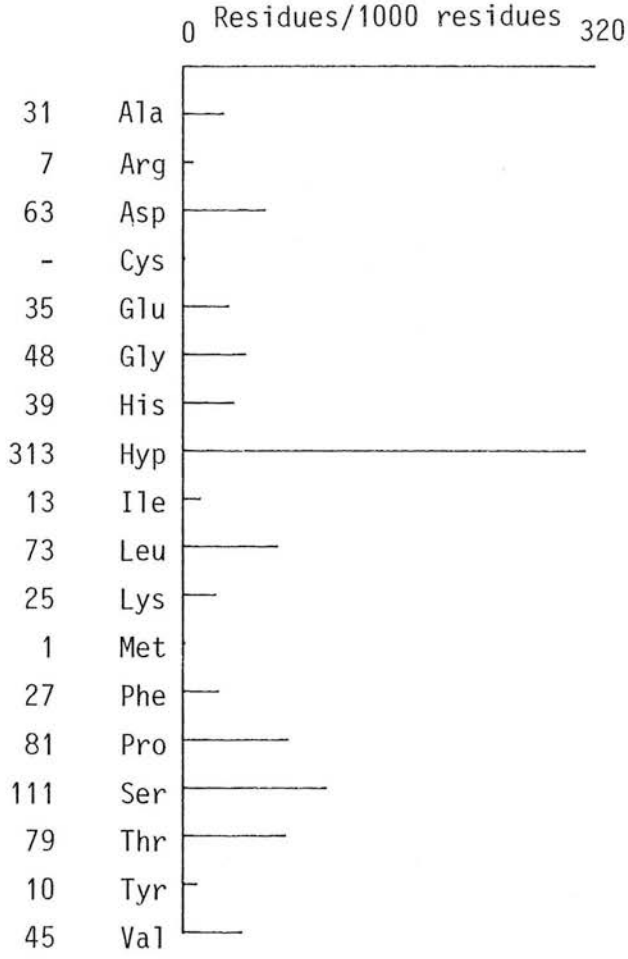
Leucaena gum



Major amino acids : Hyp Ser Pro

NOTE: key to amino acids is shown in Figure IV.2.

Gum arabic



Major amino acids : Hyp Ser Pro

FIGURE IV.2 : Key to amino acids

Ala	-	Alanine
Arg	-	Arginine
Asp	-	Aspartic acid
Cys	-	Cystine
Glu	-	Glutamic acid
Gly	-	Glycine
His	-	Histidine
Hyp	-	Hydroxyproline
Ile	-	Isoleucine
Leu	-	Leucine
Lys	-	Lysine
Met	-	Methionine
Phe	-	Phenylalanine
Pro	-	Proline
Ser	-	Serine
Thr	-	Threonine
Tyr	-	Tyrosine
Val	-	Valine

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SECTION V

ANALYTICAL STUDIES TO ESTABLISH THE PRESENCE OR ABSENCE OF GUM ARABIC IN FAECES AFTER INGESTION BY THE RAT

V.I INTRODUCTION

V.I(a) Gum arabic

Gum arabic is defined for foodstuffs purposes by all international regulatory bodies as the dried exudate obtained from the stems and branches of *Acacia senegal* (L.) Willdenow or from related species of *Acacia* (family *Leguminosae*, sub-family *Mimosoideae* (1). There are more than 900 species of *Acacia* distributed throughout tropical and sub-tropical areas of the world, but almost all (>90%) commercial gum arabic is derived from *Acacia senegal*. Only one other species, *Acacia seyal* (not closely related botanically to *Acacia senegal* and therefore not permitted in foodstuffs) yields gum in commercially useful quantities for technological, as opposed to foodstuffs, purposes. Almost all of the commercial supplies of gum arabic is produced in the sub-Sahara or Sahel zone of Africa and more than 90% of it is collected from cultivated trees in "gum gardens" from state forests (2). The gum-producing countries in order of importance are the Republic of Sudan, Senegal, Mauritania, Nigeria, Tanzania, Morocco, Ethiopia and the Republic of Somalia. The major gum markets, which buy 76% of the world's supply of gum arabic, are U.S.A., U.K., Italy, West Germany, Japan, France, Belgium and the Netherlands (3).

Gum arabic has a highly branched structure and is the half-neutralised salt of an acidic polysaccharide containing calcium, magnesium, sodium, potassium and other cations (e.g. iron, man-

ganese, zinc). The gum polysaccharide is associated with enzymes (oxidases and peroxidases) (3) and small amounts of protein (4). Complete hydrolysis with dilute acid yields D -galactose, L -arabinose, L -rhamnose and D -glucuronic acid. Within the polysaccharide field, gum arabic is unique in having high solubility forming aqueous solutions up to a concentration of 50% (w/v). Its weight average molecular weight ranges between circa 0.5×10^6 and 1.4×10^6 daltons (4).

V.I (b) Uses of gum arabic

Commercially, good quality (i.e. clean and pale in colour) gum arabic is used particularly in the pharmaceutical, cosmetic and food industries, whereas poorer darker commercial grades are used in lithography, paints and inks, foundry sands and ceramics etc. Its wide use in foodstuffs arises because of its properties as an emulsifier, stabiliser and thickener (5).

In the cosmetic industry, gum arabic is used in lotions and protective creams as it stabilises emulsions and increases their viscosities. In the pharmaceutical industry, gum arabic acts as a binder in tablets and pills. It is used in laxatives and to suspend poorly soluble, medicinal substances e.g. fat-soluble vitamins.

The main use of gum arabic is in the food industry, where it is used to influence the viscosity, body and texture of foods. Gum arabic imparts certain properties to food that cannot be obtained

from other types of materials. It is odourless, colourless, tasteless and completely water-soluble and does not affect the flavour, colour or odour of other food ingredients.

Specific examples of the uses of gum arabic in the food industry: (i) it prevents ice crystal formation by absorbing and binding water (ii) it emulsifies, thus causing the uniform distribution of fat throughout a product and (iii) it is used as an adhesive in glazes and toppings. In all these applications, gum arabic is only one of several polysaccharides that can be utilised; the choice of which emulsifier, stabiliser and viscosifier is to be used in a product is largely a matter of their relative cost-effectiveness, commercial availability etc (3).

V.I (c) A short review of dietary studies of gum arabic in animals and in Man

It was reported in 1941 and 1949 that chronic exposure to gum arabic could lead to asthma in the printing industries (6,7). Since then the allergenicity and immunological responses of gum arabic have been subjected to intensive study. A preliminary report of that work (8) has shown that gum arabic is a mild allergen in laboratory animals but its effect is less than that caused by common foodstuff components e.g. hen's egg ovalbumin.

In 1954, Mantell reported (9) that studies with guinea pigs and rabbits indicated partial digestibility of gum arabic. Gum ara-

bic was reported in 1957 (10) to have no effect on cholesterol excretion in rats. The digestibility of gum arabic was quoted to be 80% by Shue et al. (11) after they had fed rats with a diet containing 16% gum arabic and compared weights of control and test faeces. Booth et al. reported (12) that when rats were fed gum arabic in the diet at 15%, it resulted in cathartic effects including bulky, stringy faeces.

Vohra and Kratzer (13) concluded that gum arabic, fed to chicks in a nutritionally-balanced diet at a level of 2% (w/w), did not affect their growth.

Tsai et al. (14) fed gum arabic to rats at concentrations of 5% and 7% and studied the resulting effects on serum, liver and tissue cholesterol levels. Gum arabic did not decrease serum cholesterol levels but in some experiments increased liver and tissue cholesterol level, apparently expanding the body cholesterol pool.

Kelley and Tsai (15) reported that gum arabic impaired dietary cholesterol absorption but had no effect on serum and tissue cholesterol levels; apparently gum arabic increases cholesterogenesis.

Gum arabic was noted by Gohl and Gohl (16) to cause caecal distension in rats and to retard the passage of barley digesta, resulting in increased faecal bulk and water content and in the pro-

duction of lighter stools.

Kelley et al. (17) carried out further studies on the effect of gum arabic on cholesterol absorption in rats. They observed that gum arabic decreased cholesterol absorption and increased cholesterol biosynthesis in rats fed a cholesterol-containing diet but had no effect in a cholesterol-free diet. The cholesterol turnover was unaffected.

In 1981, Mclean Ross et al. (18) reported indicators of bacterial activity in the colon and caecum, when gum arabic was included in the diet of laboratory rats. They concluded that degradation of gum arabic occurs in the caecum and is associated with increased methane and volatile fatty acid production and in changes in the relative proportions of faecal volatile fatty acids.

Elsenhans et al. (19) carried out a similar study to that of Mclean Ross et al. (18) in 1981. Young Wistar rats were fed gum arabic for 7 to 8 weeks at concentrations of 0%, 10%, 20% and 40% (w/w). Gum arabic increased the small intestinal length by up to 30% without altering the mucosal protein and DNA per unit length and also increased caecal weight. The increase in caecal weight is probably an indicator of the accessibility of gum arabic to microbial degradation (19).

Young Wistar rats were fed (20) gum arabic at concentrations of 1%, 2%, 4%, 8% and 20% (w/w) (2 control groups) for 20 weeks.

Body weights, food and water consumption, urinalysis, liver, kidney weights, clinical chemistry, haematology and histology were studied. No untoward effects were observed at levels shown by calculation to be below those causing a dietary imbalance involving protein deficiency. At top dose levels, female rats showed reduced kidney weight, caecal enlargement and changes in serum urea and total carbon dioxide. The male rats exhibited no differences compared with the control rats, at concentrations up to 8%, but food and water consumption, body weight, liver and kidney weights all decreased significantly. There were no histological changes and no significant changes in haematological parameters noted in the rats at the top dose used. The no-untoward-effect concentrations were 8.6% (5.2g/kg/day) and 18.1% (13.8g/kg/day) for male and female rats respectively (20).

Mclean Ross et al. (21) reported a preliminary study of the dietary effects of gum arabic in Man in 1982. The gum appeared to be degraded in the human alimentary tract as a result of bacterial action. Gum arabic ingestion appeared to decrease serum cholesterol levels but not faecal weight or constituents. The main report of this study was published in 1983 (22). It involved the administration of gum arabic to male volunteers on a normal diet for three weeks. Little effect on glucose tolerance or stool weight was noted but serum cholesterol was decreased. There were no significant increases in bile acids or neutral sterols. Breath hydrogen increased only after chronic administration of gum arabic. The gum could not be recovered from stools, suggesting complete decomposition of gum

arabic. Faecal wet weight and dry weight increased slightly. The chronic increase in hydrogen excretion suggests that bacterial metabolism may be altered by gum arabic entering the caecum.

Fifty male and fifty female rats and mice were given 2.5% and 5.0% (w/w) gum arabic for 103 weeks. No histopathological effects were observed and there were no differences in survival between control and test animals (23).

The continued use of gum arabic in foodstuffs, world-wide, has been assured for at least the immediate future as a result of decisions taken by the Joint (FAO/WHO) Expert Committee on Food Additives (JECFA) at its meeting in Rome in May 1982 when gum arabic was placed in the category for which the acceptable daily intake (ADI) is 'not specified', a classification awarded only to substances considered to be extremely safe toxicologically. The reports available for evaluation by JECFA included the sub-acute toxicity studies in laboratory rats and a study of the dietary effects of gum arabic in Man carried out in Edinburgh University under industrial sponsorship (18, 20-22) and a long term study of gum arabic in rats conducted by the Food and Drug Administration in the U.S.A. (23).

V.1 (d) Conclusion and aims

Most of the reports cited above have been concerned with the effect of gum arabic on the health of animals and Man. It has been ascertained that gum arabic, at the concentrations used in

foodstuffs, is not toxic to either animals or to Man. The aims of the work undertaken in this part of the Thesis were:

(i) to feed gum arabic to rats, incorporated in (a) a standard small animal diet and in (b) a low residue elemental diet in order to attempt to extract undegraded gum or degradation products from faeces.

(ii) to carry out physico-chemical analyses of the faecal extracts obtained to determine the fates of the gum arabic ingested.

V.2 METHODS AND MATERIALS

(i) All the animals used in the following experiments were adult Wistar rats provided by the Animal Unit, Western General Hospital, Edinburgh.

The gum arabic, obtained from Rowntree Mackintosh Ltd., York, conformed in all respects to the British Pharmacopoeia specifications and also to the JECFA and EEC foodstuffs specifications for gum arabic (E414) (24).

(ii) Diets

(a) In the experiments in which a standard Spratts (Spillers) small animal diet was used for the control animals, gum arabic was incorporated in specified amounts for the test animals. In all cases, water was added to the diet, or diet plus gum arabic, to produce a thick paste in order to minimise losses due to scattering etc.

(b) Gum arabic was incorporated into a low residue, nutritionally complete, elemental diet ("Flexical"; Mead Johnson Laboratories, Slough). The basic diet for control animals consisted of "Flexical" powder (337.5g), gelatin (67.5g) and water (1238 ml); these components were mixed together, poured into trays (30) and allowed to set. Each rat was given the contents of one tray per day. For the test animals, gum arabic was added to the above mixture in

the quantities specified below.

(iii) Feeding method

Test rats were fed a control diet (without gum arabic) for three days, and a diet supplemented with gum arabic in the specified proportion for a further seven days; this was followed by a collection of faeces for three days. Control rats were fed the respective diet without gum arabic for seven days, followed by a collection of faeces for three days. The food containers were fixed to the cages in positions such as to minimise the contamination of faeces with uneaten food.

(iv) Collection of faeces

Faeces were collected, daily for three days, into jars containing distilled water plus crystals of thymol to reduce bacterial growth. The jars were stored in a refrigerator. Care was taken not to collect faeces observed to be admixed with scattered food debris.

(v) Extraction method

The faeces/distilled water/thymol mixture contained in two stout, plastic bags, was put into a 'stomacher' to give a mixture of uniform consistency. More distilled water was added and the mixture was left overnight in the cold room. The faecal mixture was centrifuged for 20 minutes using a 2L MSE Mistral centrifuge at 7000g.

The supernatant was removed and dialysed against running tap water for two days and against distilled water for a further two days. Half the dialysate was freeze-dried. The other half of the dialysate was precipitated with 4 volumes of ethanol containing 1% (v/v) concentrated HCl. It was left overnight in the refrigerator.

The precipitate was vacuum filtered using Whatman filter papers No 41 and No 1, and washed with ethanol. The precipitate was dissolved in distilled water and the solution dialysed as before. The dialysate was freeze-dried.

(vi) Analysis of extracts

The resulting extracts were analysed using some of the methods outlined in Section II.

(vii) Summary of experiments

(a) A 5% (w/v) aqueous solution of gum arabic was prepared and put through the extraction procedure from the dialysis stage onwards;

(b) 20 rats were fed the standard diet and the elemental diet respectively and the faeces obtained were treated as in (v);

(c) 10 rats were fed the standard diet and the elemental diet respectively and gum arabic was mixed with the

resultant faeces and re-extracted as in (v);

(d) using the standard diet, gum arabic was fed to two groups, each of 10 rats, at concentrations of (i) 2g/day/rat and (ii) 4g/day/rat and the faeces obtained were treated as in (v);

(e) using the elemental diet, gum arabic was incorporated at concentrations of 2g/day/rat for a group of 20 rats and at 1g/day/rat for a group of 20 rats and the faeces obtained were treated as in (v).

V.3 RESULTS

The analytical data for gum arabic and the faecal extracts obtained from the animal feeding experiments are detailed in Tables V.1, V.2, V.3 and V.4. Figures V.1, V.2 and V.3 contain the amino acid profiles for: the freeze-dried faecal extracts obtained when rats were fed the standard diet (i) alone and (ii) including gum arabic at 2g/day/rat; gum arabic; gelatin and the freeze-dried faecal extracts obtained when rats were fed the elemental diet including gum arabic at 2g/day/rat and 1g/day/rat respectively.

V.4 DISCUSSION

In this study, gum arabic was incorporated into two different

diets which were subsequently fed to adult Wistar rats; 1) a standard Spratts (Spillers) small animal diet containing protein, fat, minerals, vitamins, carbohydrate and fibre and 2) a low residue elemental diet "Flexical".

Elemental diets are semi-synthetic fibre-free liquid diets containing a full range of basic nutrients. Carbohydrate, protein and fat are presented to the gastro-intestinal tract in a form that does not require intact digestive capabilities. Elemental diets do not have an effect on the types of bacteria present but decrease the overall bacterial mass present in intestinal contents and reduce stool weight (25).

Preliminary experiments were carried out to ascertain the most effective methods concerning (1) the preparation and mode of presentation of the diets, with and without gum arabic, to the rats (2) the collection of faeces and (3) the extraction of gum arabic from faeces.

For the purposes of comparison, extracts were isolated, after the initial dialysis stage, by two methods; (i) by precipitation with acidified ethanol and (ii) by freeze-drying.

The standard diet was used in the first set of experiments. Gum arabic was treated in the same manner as faeces and the yield was 82%. Losses were likely to have occurred because of the lengthy procedure involved in the treatment. When gum arabic was mixed with

control faeces and re-extracted, only 56% gum arabic was recovered. This rather low yield may be accounted for by the mechanical losses involved in extracting the gum from faeces, the homogenisation and centrifugation stages, and by the possibility of gum arabic degradation having taken place when the gum was in contact with faecal bacteria.

The analytical data for re-extracted gum arabic were compared with those for gum arabic alone in Table V.1. The ash contents were similar with values of 2.9% and 3.8% and 3.4% and 3.7%; the nitrogen contents increased from 0.29% and 0.24% to 0.98% and 1.50%; intrinsic viscosities increased from 16 $ml g^{-1}$ and 17$ml g^{-1}$ to 26 $ml g^{-1}$ and 33 $ml g^{-1}$ and the specific rotations decreased from -29^{o} and -29^{o} to -26^{o} and -24^{o}.

The nitrogen contents of re-extracted gum arabic would be expected to increase due to bacterial debris and gut mucoproteins present in faeces. The increased intrinsic viscosities and decreased specific rotations probably arose because the re-extracted gum arabic was associated with impurities.

Control faeces (Table V.2) gave extracts (total weight 1.7g) of high ash content (6.2%), high nitrogen contents (6.01% and 8.34%), high intrinsic viscosity (160 $ml g^{-1}$) and highly positive specific rotation. It was not possible to obtain an exact value for the specific rotation because of the cloudy solution obtained with this extract.

TABLE V.1 : Analytical data for (i) gum arabic and (ii) gum arabic mixed with faeces and re-extracted (rats fed the standard diet).

	(i) GUM ARABIC		(ii) FAECAL EXTRACTS (gum arabic)	
	AP	FD	AP	FD
Total yield, %	82		56	
Moisture, %	10.5	11.1	4.1	6.4
Ash, %[a]	2.9	3.8	3.4	3.7
Nitrogen, %[a]	0.29	0.24	0.98	1.50
Intrinsic viscosity,[a] mlg^{-1}	16	17	26	33
Specific rotation,[a] degrees	-29	-29	-26	-24

Footnotes : a = corrected for moisture content

AP = precipitated with 4 volumes of ethanol containing 1% (v/v) concentrated HCl

FD = freeze-dried

TABLE V.2 : Analytical data for (i) faecal extracts from control rats fed the standard diet (ii) test rats fed with gum arabic incorporated at 2g/day/rat and 4g/day/rat respectively.

	CONTROL FAECAL EXTRACTS		TEST FAECAL EXTRACTS (Gum arabic at 2g/day/rat)		TEST FAECAL EXTRACTS (Gum arabic at 4g/day/rat)	
	AP	FD	AP	FD	AP	FD
Total weight of extracts (g)	1.7		2.1		2.0	
Moisture, %	10.7*	10.1	10.7*	12.9	10.7*	9.0
Ash, %[a]	nd	6.2	nd	4.5	nd	4.6
Nitrogen, %[a]	6.01	8.34	6.60	10.87	8.82	10.34
Intrinsic viscosity[a] $ml g^{-1}$	nd	160	265	180	135	130
Specific rotation[a] degrees	nd	Highly positive	nd	Highly positive	nd	Highly positive

Footnotes : a = corrected for moisture content

AP = precipitated with 4 volumes of ethanol containing 1% (v/v) concentrated HCl

FD = freeze-dried

nd = not done due to lack of sample

* = estimated

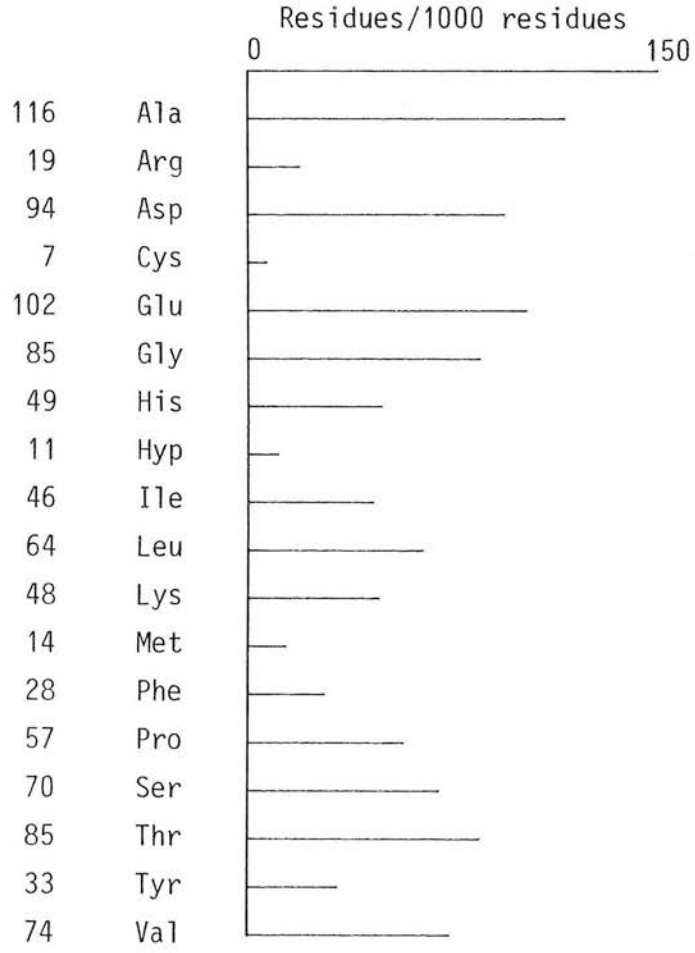
The analytical data for the test faecal extracts isolated when gum arabic was incorporated in the standard diet at 2g/day/rat and 4g/day/rat are detailed in Table V.2. Nitrogen contents (6.60%, 10.87% and 8.82%, 10.34%) and intrinsic viscosities (265 $ml g^{-1}$, 180 $ml g^{-1}$ and 135 $ml g^{-1}$, 130 $ml g^{-1}$) respectively, were greater than those (Table V.1) for re-extracted gum arabic (nitrogen contents, 0.98%, 1.50% and intrinsic viscosities 26 $ml g^{-1}$, 33 $ml g^{-1}$) but similar to the values for the control faecal extracts (nitrogen contents 6.01%, 8.34% and intrinsic viscosity 160 $ml g^{-1}$). It was not possible to obtain exact values for the specific rotations for the test faecal extracts due to the cloudiness of the resultant mixture, but their optical rotations were highly positive and similar to that of the control faecal extract.

The total weights of extracts isolated in all three cases from; faeces alone and faeces where gum arabic was incorporated in the diet at 2g/day/rat and 4g/day/rat were similar (1.7g, 2.1g and 2.0g respectively).

Gum arabic and the faecal extracts obtained when rats were fed the standard diet (i) alone and (ii) including gum arabic at 2g/day/rat, were submitted to amino acid analysis; the resulting profiles are compared in Figures V.1 and V.2. The three major amino acids in (i) were alanine, glutamic acid and aspartic acid and in (ii) were aspartic acid, alanine and glutamic acid. No major differences were found between these two profiles which are very different from that of gum arabic (Figure V.2), in which the major

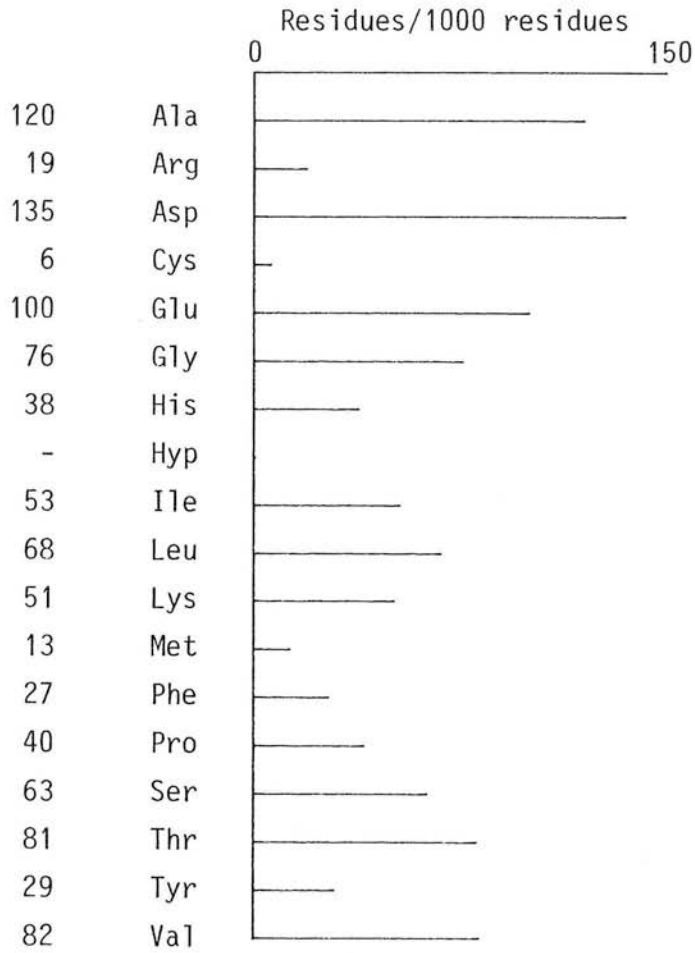
FIGURE V.1 : Amino acid profiles

FD faecal extract (rats fed standard diet)



Major amino acids : Ala Glu Asp

FD faecal extract (rats fed standard diet including gum arabic at 2g/day/rat

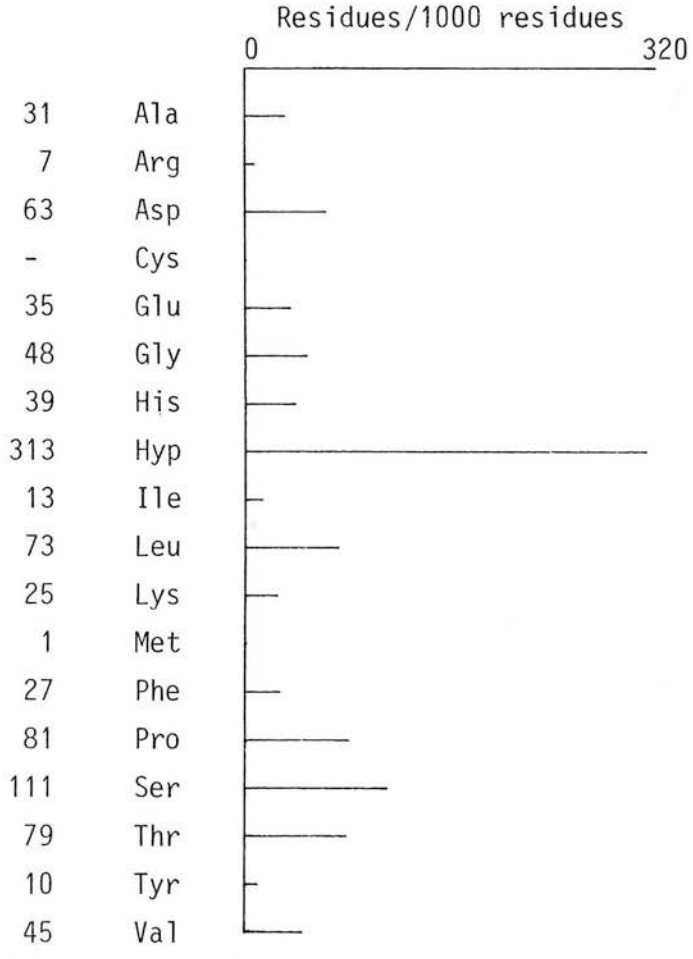


Major amino acids : Asp Ala Glu

Footnote : key to amino acids in Figure IV.2. FD : freeze-dried.

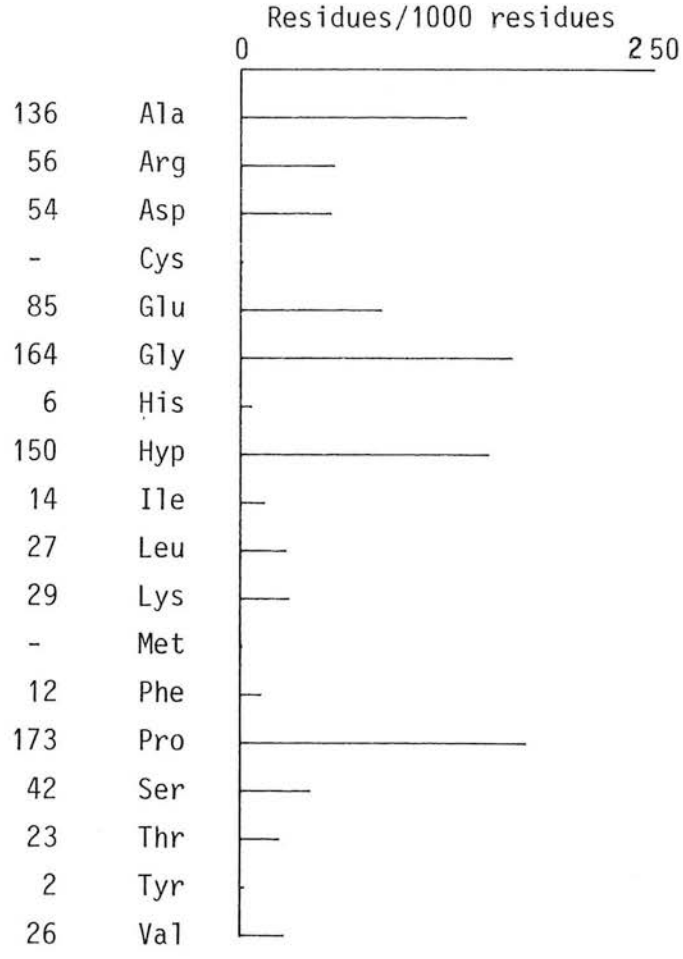
FIGURE V.2 : Amino acid profiles

Gum arabic



Major amino acids : Hyp Ser Pro

Gelatin



Major amino acids : Pro Gly Hyp

Footnote : key to amino acids in Figure IV.2.

amino acids are hydroxyproline, serine and proline. It was important to note that the prominent hydroxyproline peak in the profile of gum arabic, is absent in the profile of the test faecal extract and very small in the profile of the control faecal extract.

These results indicate that gum arabic was degraded on incorporation into a standard diet fed to rats, irrespective of the dose levels used here.

The elemental diet "Flexical" was used in the second set of experiments. Gum arabic, treated in the same manner as faeces, gave a yield of 82%. Losses were likely to have occurred due to the lengthy procedure involved in the treatment. When gum arabic was mixed with control faeces and extracted, the yield was 65%. The low yield may be accounted for by the mechanical losses involved in extracting the gum from faeces, the homogenisation and centrifugation stages, and by the possibility of gum arabic degradation having taken place when the gum was in contact with faecal bacteria.

The analytical data for re-extracted gum arabic were compared with those for gum arabic alone in Table V.3. The ash contents were similar with values of 2.9% and 3.8% and 3.0% and 3.4%; nitrogen contents increased from 0.29% and 0.24% to 0.33% and 0.72%; intrinsic viscosities increased from 16 $ml\,g^{-1}$ and 17 $ml\,g^{-1}$ to 20 $ml\,g^{-1}$ and 19 $ml\,g^{-1}$; specific rotations decreased from -29^{o} and -29^{o} to -27^{o} and -27^{o} and the molecular weights decreased from 1.0×10^{6} and 0.9×10^{6} to 0.5×10^{6} and 0.5×10^{6}.

TABLE V.3 : Analytical data for (i) gum arabic and (ii) gum arabic mixed with faeces and re-extracted (rats fed the elemental diet).

	GUM ARABIC		FAECAL EXTRACTS (gum arabic)	
	AP	FD	AP	FD
Total yield, %	82		65	
Moisture, %	10.5	11.1	7.7	6.4
Ash, %[a]	2.9	3.8	3.0	3.4
Nitrogen, %[a]	0.29	0.24	0.33	0.72
Intrinsic viscosity, $ml\,g^{-1}$[a]	16	17	20	19
Specific rotation degrees[a]	-29	-29	-27	-27
Molecular weight $\bar{M}_w \times 10^6$ [a]	1.0	0.9	0.5	0.5

Footnotes : a = corrected for moisture content

AP = precipitated with 4 volumes of ethanol containing 1% (v/v) concentrated HCl

FD = freeze-dried

The increased nitrogen contents were a result of contamination from nitrogenous material already present in faeces. The decreased specific rotations and more particularly decreased molecular weights indicate some form of gum arabic degradation which could possibly have arisen from contact with faecal bacteria.

Control faeces were extracted and yielded material (total weight 0.2g) which was analysed as far as possible; the results are shown in Table V.4. The nitrogen contents (10.57% and 12.07%) and intrinsic viscosity (150 $ml g^{-1}$) were high compared to those for re-extracted gum arabic (Table V.3).

Gum arabic was incorporated in the elemental diet at a dose level of 2g/day/rat. Material (total weight 3.0g) isolated from faeces was analysed and the data (Table V.4) compared with those for re-extracted gum arabic (Table V.3). Ash contents were similar with values of 3.0% and 3.4% and 3.6% and 3.1%; nitrogen contents increased from 0.33% to 0.68% and decreased slightly from 0.72% to 0.69%; intrinsic viscosities decreased slightly from 20 $ml g^{-1}$ and 19 $ml g^{-1}$ to 14 $ml g^{-1}$ and 15 $ml g^{-1}$; specific rotations decreased slightly from -27^{o} and -27^{o} to -22^{o} and -22^{o} and molecular weights decreased slightly from 0.5×10^{6} and 0.5×10^{6} to 0.4×10^{6} and 0.3×10^{6}.

Gum arabic was then incorporated in the elemental diet at a reduced dose level of 1g/day/rat. Material (total weight 1.4g) extracted from faeces was analysed and the data are shown in Table

TABLE V.4 : Analytical data for faecal extracts from rats fed the elemental diet, alone and with gum arabic incorporated, firstly at 2g/day/rat and secondly at 1g/day/rat.

	CONTROL FAECAL EXTRACTS		TEST FAECAL EXTRACTS (Gum arabic at 2g/day/rat)		TEST FAECAL EXTRACTS (Gum arabic at 1g/day/rat)	
	AP	FD	AP	FD	AP	FD
Total weight of extracts (g)	0.2		3.0		1.4	
Moisture, %	12.1*	12.1*	10.9	12.6	12.1*	12.9
Ash, %[a]	nd+	nd+	3.6	3.1	nd+	4.5
Nitrogen, %[a]	10.57	12.07	0.68	0.69	6.90	10.10
Intrinsic viscosity $ml g^{-1}$[a]	nd+	150	14	15	240	250
Specific rotation degrees,[a]	nd+	nd+	-22	-22	nd+	nd++
Molecular weight $\overline{M}_W \times 10^6$[a]	nd+	nd+	0.4	0.3	nd+	nd++

Footnotes : a = corrected for moisture content

AP = precipitated with 4 volumes of ethanol containing 1% (v/v) concentrated HCl

FD = freeze-dried

nd+ = not done due to lack of sample

nd++ = not done due to cloudiness of solutions

* = estimated

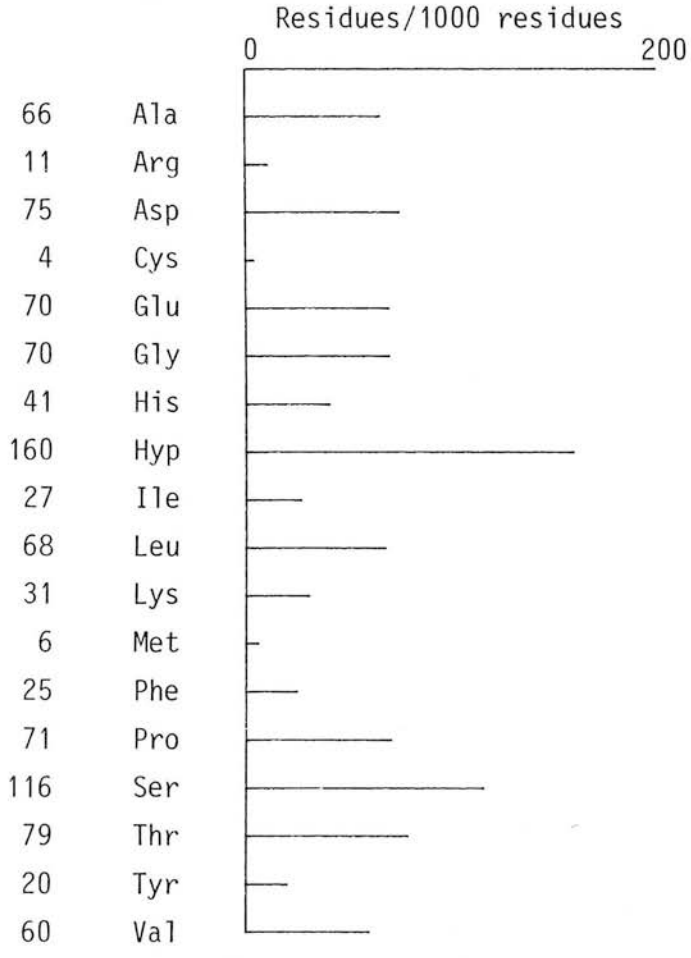
V.4. The nitrogen contents (6.90% and 10.90%) and intrinsic viscosities (240 $ml g^{-1}$ and 250 $ml g^{-1}$) were very different from those for the faecal extracts obtained when gum arabic was incorporated in the diet at 2g/day/rat (Table V.4) but were similar to the data for the control faecal extracts (nitrogen contents 10.57% and 12.07% and intrinsic viscosity 150 $ml g^{-1}$).

When gum arabic was incorporated in the elemental diet at a dose level of 2g/day/rat, the faecal extracts isolated (Table V.4) appeared to be partially degraded gum arabic. Halving the dose level of gum arabic to 1g/day/rat produced faecal extracts giving analytical data (Table V.4) different from those for gum arabic (Table V.3) and from those for the apparently partially degraded gum arabic. The difference in weights between the control faecal extracts (total weight 0.2g) and the test faecal extracts (total weight 1.4g) where the gum arabic dosage used was 1g/day/rat, was considered to be due to the presence of gum arabic degradation products in faeces.

Gum arabic, gelatin and the faecal extracts obtained when gum arabic was incorporated in the elemental diet at (1) 2g/day/rat and (2) 1g/day/rat were submitted to amino acid analysis. The profiles are shown in Figures V.2 and V.3. The three major amino acids in gum arabic were hydroxyproline, serine, and proline, in gelatin were proline, glycine and hydroxyproline; in the faecal extract from (1) were hydroxyproline, serine and threonine; and in the faecal extract from (2) were alanine, aspartic acid and glutamic acid. The amino

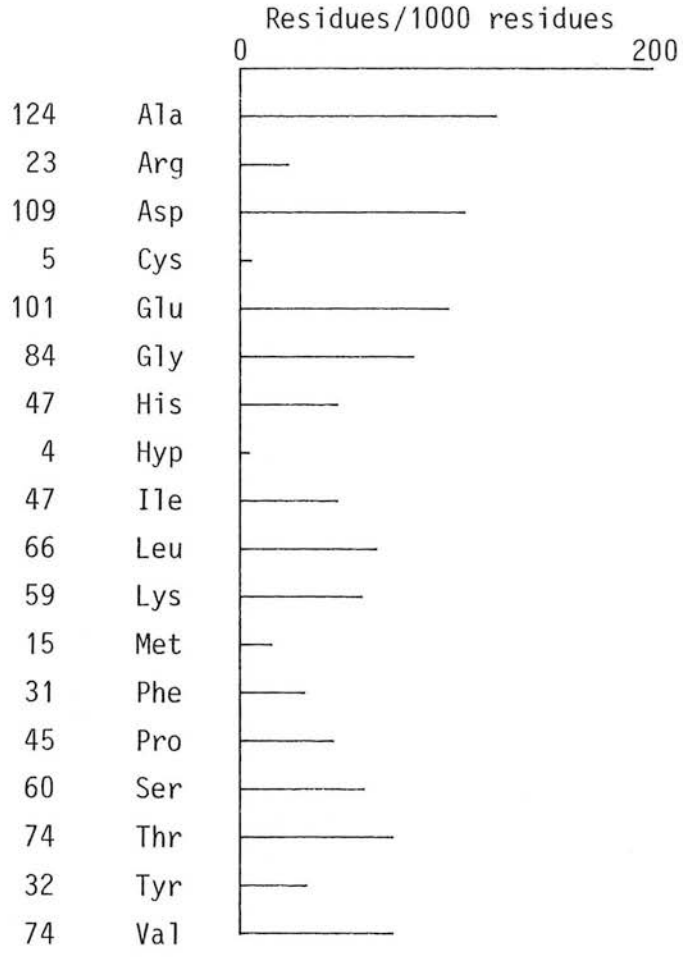
FIGURE V.3 : Amino acid profiles

FD faecal extract (rats fed elemental diet including gum arabic at 2g/day/rat)



Major amino acids : Hyp Ser Thr

FD faecal extract (rats fed an elemental diet including gum arabic at 1g/day/rat)



Major amino acids : Ala Asp Glu

Footnote : key to amino acids in Figure IV.2. FD : freeze-dried

acid profile for gelatin showed that the increased nitrogen content of the faecal extracts was not due to undigested gelatin. The predominant amino acids in both gum arabic and the faecal extract from (1) were hydroxyproline and serine. However, the prominent hydroxyproline peak in gum arabic was reduced by approximately 50% in the faecal extract from (1) and was very small in the faecal extract from (2).

The amino acid information also indicates that the degradation of gum arabic included in the elemental diet appears to be dose-related.

Analytical data for the material isolated by precipitation with acidified ethanol did not differ appreciably from those for the freeze-dried material. One consistent feature was that the nitrogen contents of the faecal extracts tended to be greater in the freeze-dried material due to the fact that the acidified ethanol precipitates would be expected to be less contaminated with impurities than the freeze-dried extracts.

It has been reported that gum arabic is degraded by colonic bacteria (18, 21, 22). Gum arabic also appears to be degraded by rat faecal bacteria.

Simple sugars and disaccharides are absorbed efficiently as they pass through the small intestine. So it is likely that the major sources of carbon and energy for saccharolytic colonic bacteria

are complex carbohydrates. These include complex carbohydrates produced by hosts such as mucin (saliva), mucin produced by goblet cells lining the intestine, cell surface glycoproteins from sloughed epithelial cells and complex carbohydrates from the diet such as gum arabic (26).

The strains of anaerobic bacteria found to ferment the widest range of polysaccharide substrates are in the two genera Bacteroides and Bifidobacterium. Gum arabic is known to be fermented by strains of Bifidobacterium (27,28).

Under normal circumstances, the intestinal tract, on a standard diet, contains a rapidly growing and metabolising microbial population of high density, which is nourished and sustained by a continuous supply of nutrients derived both from ingested food and from body secretions. The intestinal microflora population may include bacterial types that can both synthesise and utilise nutrients and hence can exert a significant influence on the nutritional requirements of the host. These bacteria adhere to the irregular moist intestinal wall in large numbers and serve as an inoculum for each new supply of substrate entering the intestinal tract. The organisms that survive, die and multiply are governed, to a large extent, by the composition of the substrate and by the environment that the microbes themselves produce (29).

In an elemental diet the situation is changed. The quantity of nutrients reaching the bacteria in the intestinal tract will be

reduced causing a decrease in the survival rate of the micro-organisms. This would lead to reduced bacterial mass. The decrease in the bacterial population will lead to the reduced utilisation of nutrients. This would provide an explanation for the dose-related effect on the extent of degradation of gum arabic when incorporated into the elemental diet "Flexical".

The experiments undertaken with gum arabic and reported in this Thesis were completed in 1983. Further studies have been completed and published by other workers since then, as outlined below.

Mclean Ross et al. (30) have confirmed that gum arabic degradation takes place in the rat caecum. When gum arabic was incorporated in the rat's diet, it was possible to precipitate gum arabic using acidified ethanol, along the gastro-intestinal tract as far as the terminal ileum; but none however, was found in the caecum, colon or rectum. Measurements of methane and hydrogen in the expired breath and of volatile fatty acids are indices of metabolic activity in the large intestine. All rats from the age of 3 months produce methane in their expired breath and hydrogen is excreted throughout their lifetime. Caecectomy abolished hydrogen production which indicated that the caecum is the major site of bacterial activity. Caecectomy resulted in the loss of these bacteria and hence there was no gum arabic degradation (30).

Adult male and female rats were fed a synthetic diet containing 10% gum arabic or cellulose. There were increased concentra-

tions of total volatile fatty acids (acetate, propionate and butyrate) in caecal fluid and hepatic portal venous plasma of both sexes. This increase was a result of substantial fermentation in the large bowel (31).

Mallett et al. (32) reported that gum arabic (50g/kg diet) given to weanling rats for 4 weeks caused an increase in the weight of caecal wall and caecal contents and also increased the activity of certain bacterial enzymes e.g. nitro reductase and nitrate reductase, indicating that enzyme induction was taking place. Conning et al. (33) also observed that when rats had gum arabic incorporated in a fibre-free diet, caecal size and the activities of reductase enzymes increased.

These reports demonstrate that gum arabic influences the metabolic activity of rat caecal microflora.

V.5 CONCLUSIONS

(1) Gum arabic was degraded on incorporation in the standard diet at dose levels of 2g/day/rat and 4g/day/rat.

(2) Gum arabic was partially degraded on incorporation in the elemental diet "Flexical" given to rats at a dose level of 2g/day/rat and degraded more extensively at a reduced dose level (1g/day/rat).

(3) Gum arabic mixed with faeces from rats fed the elemental diet "Flexical" was partially degraded by faecal bacteria.

(4) The different results obtained when gum arabic was incorporated into standard and elemental diets indicate the importance of the type of diet and dose-level used in animal dietary studies.

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SECTION VI

AN ANALYTICAL ATTEMPT TO ESTABLISH THE EXTENT OF DEGRADATION (IF ANY) OF GUM KARAYA AFTER INGESTION BY THE RAT

VI.1 INTRODUCTION

VI.1(a) Gum karaya

Gum karaya is defined for trade and legislative purposes as the dried gummy exudate from Sterculia species (Family Sterculiaceae), a family of deciduous trees growing in the dry elevated regions of North and Central India, China, Indochina, Sudan, Australia, Senegal and New Guinea.

Gum karaya exudes naturally, but most of it is produced commercially in India in response to tree tapping procedures. The gum is produced rapidly, most of it in about a day. The major Indian contributing species are Sterculia urens and Sterculia villosa; very small quantities of gum are obtained from Sterculia setigera in Africa (1). It has been established that the gum exudates from these three species are very similar in composition and physicochemical properties (2).

The exudate from Cochlospermum gossypium (Family Bixineae), a small deciduous tree which grows in the North-west Himalaya, resembles Sterculia and has been termed gum karaya for trade purposes although it is chemically different (3).

Gum karaya is a complex, partially acetylated polysaccharide of high molecular weight. The acetic acid is labile. Gum karaya is rich in calcium, magnesium and potassium salts and contains D

-galacturonic acid, D -glucuronic acid, D -galactose and L -rhamnose. It does not dissolve readily in water but swells to give a viscous colloidal solution (4,5).

VI.1(b) Uses of gum karaya

Gum karaya is used in the textile, cosmetic, food and pharmaceutical industries as a thickener, emulsifier and stabiliser (6).

Gum karaya finds a very important use in laxative preparations, dental fixatives and as an adhesive in colostomy appliances. In the food industry, it is used in whipped cream products, ice cream, sauces and many other processed foods (7,8).

VI.1(c) A short review of dietary studies of gum karaya in animals and in Man

In 1938 (9), gum karaya was reported to be undegraded when ingested by dogs although faecal nitrogen excretion was increased. Gum karaya did not affect starch digestion and vitamin A utilisation in rats. However, gum karaya was found to increase faecal bulk and moisture and to be a good laxative causing no irritation.

In 1941 Hoelzel (10) reported that rats fed a diet including granular karaya at levels of 10% and 25% (w/w) developed intestinal lesions. However, in 1948 (11) it was reported that rats fed 10%

and 25% (w/w) granular karaya developed no caecal ulcerations.

It was observed by Vohra and Kratzer (12) that when gum karaya was added to the diet of chickens at a level of 2% there was a depression in their growth.

Since that time, little has been published concerning the effect of gum karaya in the diet of animals and Man until recently.

In 1981 (13) gum karaya was used in a long term feeding study in rats. The gum was included in a fibre-free diet at concentrations of 10%, 20% and 40% (w/w). Gum karaya was found to have an apparent digestibility of less than 10%.

In 1982 (14) gum karaya was reported to give a mild immune response in mice, comparable to that of the common foodstuff's component hen's egg ovalbumin.

The fate of gum karaya in the rat was reported in 1982 (15). Rats were fed a diet containing 5% (w/w) gum karaya for 24 hours and faeces and urine were collected for a period of 72 hours. The sugar composition of the faeces was determined and compared to that of the whole gum. There was found to be good agreement between the two indicating that very little degradation of gum karaya had taken place on ingestion by the rat.

In a further study of gum karaya (16) groups of 15 rats of

each sex were given gum karaya at dietary levels of 0 (control), 0.2%, 1% and 5% (w/w) for 13 weeks. Faecal bulk increased in all treated groups throughout the study. The no-untoward effect level was 5% (w/w) of the diet providing a mean intake of about 4g gum karaya/kg body weight/day.

As a result of these studies, a temporary 'acceptable daily intake' (ADI) of 0 - 20 mg/kg/day was allocated to gum karaya by JECFA in 1983 (22).

In 1983 (17), it was reported that the digestibility of casein was reduced in the presence of gum karaya; this may be a result of interference with the enzyme complex formation in proteolytic digestion.

The only study to date concerning the effect of gum karaya on Man was reported in 1983. Five male volunteers consumed 10.5g gum karaya/day for 21 days. Measurements made before and after the test period showed that ingestion of gum karaya had no significant effect on many parameters including faecal fat, bile acids, glucose tolerance, serum cholesterol and plasma biochemistry. The daily test intake, which was large in relation to the minor amounts of gum karaya used in foodstuffs, did not cause any toxic effects in terms of the measurements made; moreover, gum karaya had no metabolic action of any consequence (18).

VI.1(d) Conclusion and aims

The previous reports indicated that gum karaya is metabolically inert in animals and in Man when incorporated in a normal diet. No attempt had been made to extract the undigested gum from faeces and to compare the extract with the original gum. Hence, the aims of the work undertaken in this section of the Thesis were:

(i) to feed gum karaya to rats incorporated in (a) a standard diet and (b) an elemental diet and attempt to recover undegraded gum or degradation products from faeces;

(ii) to carry out physico-chemical analyses of the faecal extracts obtained to determine the fate of ingested gum karaya in the rat.

VI.2 METHODS AND MATERIALS

(i) All the animals used in the following experiments were adult Wistar rats, provided by the Animal Unit, Western General Hospital, Edinburgh.

The gum karaya (E416) (8) used as the Test Article was obtained from Norgine Ltd., London, from a consignment tested and approved for routine manufacturing purposes.

(ii) Diets

(a) In the experiments in which a standard Spratts (Spillers) small animal diet was used for the control animals, gum karaya was incorporated in specified amounts for the test animals. In all cases, water was added to the diet or diet plus gum karaya, to produce a thick paste in order to minimise losses due to scattering and to assist the palatability of gum karaya.

(b) Gum karaya was incorporated in a low residue nutritionally-complete elemental diet "Flexical" (Mead Johnson Laboratories, Slough). The basic diet for the control animals consisted of "Flexical" powder (337.5g), gelatin (50.7g) and water (1238 ml); these were mixed together, poured into trays (30) and allowed to set. Each rat was given the contents of one tray per day. For the test animals gum karaya was added to the above mixture in the quantities specified below.

(iii) Feeding method

Test rats were fed a control diet (without gum karaya) for three days, and a diet supplemented with gum karaya in the specified proportion for a further seven days; this was followed by collection of faeces for three days. Control rats were fed the respective diet without gum karaya for seven days, followed by collection of faeces for three days. The food containers were fixed to the cages in positions such as to minimise contamination of the faeces with uneaten

food.

(iv) Collection of faeces

Faeces were collected daily for three days into jars containing 0.2M ammonium hydroxide plus crystals of thymol to reduce bacterial growth. Gum karaya is water-insoluble but is soluble in ammonium hydroxide. The jars were stored in a refrigerator. Care was taken not to collect faeces observed to be admixed with scattered food debris.

(v) Extraction method

The faeces/ammonium hydroxide/thymol mixture, contained in two stout plastic bags, was put into a 'stomacher' to give a mixture of uniform consistency. More ammonium hydroxide was added and the mixture left overnight in the cold room. The faecal mixture was centrifuged for 20 minutes using a 2L MSE Mistral centrifuge at 7000g. The supernatant was removed, dialysed against running tap water for two days and against distilled water for a further two days. Half the dialysate was freeze-dried. The other half was precipitated with 4 volumes of ethanol containing 1% (v/v) concentrated HCl. It was left overnight in the refrigerator.

The precipitate was vacuum filtered using Whatman filter papers No. 41 and No. 1 and washed several times with ethanol to remove the HCl. The precipitate was air dried.

(vi) Analysis of extracts

The resulting extracts were analysed using some of the methods detailed in Section II of this Thesis.

(vii) Summary of experiments

(a) A 0.4% (w/v) solution of gum karaya was prepared with ammonium hydroxide (0.2M) and put through the extraction procedure from the dialysis stage onwards;

(b) 10 rats were fed the standard diet and the elemental diet respectively. The faeces were treated as in (v);

(c) 10 rats were fed the standard diet and the elemental diet respectively and gum karaya was mixed with the faeces and re-extracted as in (v);

(d) using the standard diet, gum karaya was incorporated at a dose level of 1.2g/day/rat to 10 rats and the faeces treated as in (v);

(e) using the elemental diet, gum karaya was incorporated at a dose level of 1.2g/day/rat to 10 rats and the faeces treated as in (v).

VI.3 RESULTS

The analytical data for gum karaya and for the faecal extracts

obtained from the animal feeding studies are detailed in Tables VI.1 and VI.2. The amino acid profiles for gum karaya and for the faecal extracts from rats fed the standard diet (i) alone and (ii) with gum karaya incorporated at 1.2g/day/rat, are shown in Figure VI.

VI.4 DISCUSSION

In this study, gum karaya was incorporated into two different rat diets 1) a standard Spratts (Spillers) small animal diet containing protein, fat, minerals, vitamins, carbohydrate and fibre and 2) a low residue elemental diet, 'Flexical'.

Elemental diets are semi-synthetic fibre-free liquid diets containing a full range of basic nutrients. Carbohydrate, protein and fat are presented to the gastro-intestinal tract in a form that does not require intact digestive capabilities. They are bulk-free and have minimal residue. Elemental diets do not have an effect on the types of bacteria present but decrease the overall bacterial mass present in intestinal contents and reduce stool weight (19).

Preliminary experiments were carried out to ascertain the most efficient methods concerning (1) the preparation and mode of presentation of the diets to the rats, with or without gum karaya (2) the collection of faeces and (3) the extraction of gum karaya from faeces.

For the purpose of comparison, extracts were isolated after

the dialysis stage by two methods; (i) by precipitation with acidified ethanol and (ii) by freeze-drying.

The standard diet was used in the first set of experiments.

When gum karaya was put through the extraction procedure, the yield was 74%. The granular, natural gum karaya used contained sand, bark and other plant debris (ca. 8%) which was removed on centrifugation. Some deacetylation (5-10%) and mechanical losses (5-10%) also inevitably occurred during the extraction procedure: thus a total loss of 26% is explicable. When gum karaya was mixed with faeces and re-extracted, 60% of the gum was recovered. The further decrease in yield can be explained, at least in part to losses during centrifugation; the pellet-supernatant interface was not sharply defined and varying the time and speed of centrifugation did not improve it.

Analytical data for gum karaya and re-extracted gum karaya are compared in Table VI.1. Ash contents were similar (0.0% and 9.7% and 1.9% and 8.3%); nitrogen contents increased from 0.08% and 0.00% to 3.17% and 4.10%; intrinsic viscosities decreased from 535 mlg^{-1} and 515 mlg^{-1} to 330 mlg^{-1} and 260mlg^{-1} and specific rotations decreased from $+59^{o}$ and $+45^{o}$ to $+47^{o}$ and $+38^{o}$.

The increased nitrogen contents of the faecal extracts can only be due to the presence of nitrogeneous contaminants such as gut mucoproteins, bacterial and other debris present in faeces. The

TABLE VI.1 : Analytical data for: gum karaya; gum karaya mixed with faeces and re-extracted; faecal extracts from (i) control rats and (ii) test rats given 1.2g gum karaya/day/rat. All rats fed the standard diet.

	GUM KARAYA		FAECAL EXTRACTS (Gum karaya mixed with faeces)		CONTROL FAECAL EXTRACTS		FAECAL EXTRACTS (Gum karaya at 1.2g/rat/day)	
Total weight of extracts (g)			6.0		2.1		11.9	
Total yield, %	74		60				33	
	AP	FD	AP	FD	AP	FD	AP	FD
Moisture, %	12.5	15.3	9.5	10.2	10.3*	10.3	13.7	15.6
Ash, %[a]	0.0	9.7	1.9	8.3	nd	5.8	1.1	9.1
Nitrogen, %[a]	0.08	0.00	3.17	4.10	8.61	8.04	2.22	2.23
Intrinsic viscosity mlg^{-1}[a,c]	535	515	330	260	nd	180	250	250
Specific rotation degrees[a,b]	+59	+45	+47	+38	nd	nd+	+60	+43

Footnotes : AP : precipitated with 4 volumes of ethanol containing 1% (v/v) concentrated HCl
FD : freeze-dried
nd : not done due to small quantity of sample
nd+ : not done due to cloudiness of faecal extract solution
* : estimated
a : corrected for moisture content
b : in 0.2M ammonium hydroxide
c : in 0.1M ammonium hydroxide and 1.0M sodium chloride

differences between the intrinsic viscosities and the specific rotations for gum karaya and for re-extracted gum karaya were probably attributable to the contaminants present in the re-extracted gum.

When control faeces were extracted, the isolated material (total weight 2.1g) gave nitrogen contents of 8.61% and 8.04% and an intrinsic viscosity of 180 $ml g^{-1}$. These values (Table VI.1) differed from the data for gum karaya and for re-extracted gum karaya. Ash, intrinsic viscosity and specific rotation determinations were not possible for the control faecal extract, obtained by precipitation with acidified ethanol, because of the small quantity of sample available. The control faecal extract obtained by freeze-drying produced cloudy mixtures which were not sufficiently clarified by repeated filtration to enable the specific rotation to be determined.

When gum karaya was incorporated into the standard diet at 1.2g/day/rat, the recovery of gum from faeces was 33%, based on the weight of gum karaya incorporated into the foodstuff placed in the rats' cages. There were however, considerable losses resulting from food spillage by the rats (ca. 25%) and incomplete collection of faeces, as faeces contaminated with scattered food debris were rejected (ca. 20%).

Analytical data for the test faecal extracts from gum karaya diet supplementation and for re-extracted gum karaya are shown in Table VI.1. Ash contents were similar (1.9% and 8.3% and 1.1% and

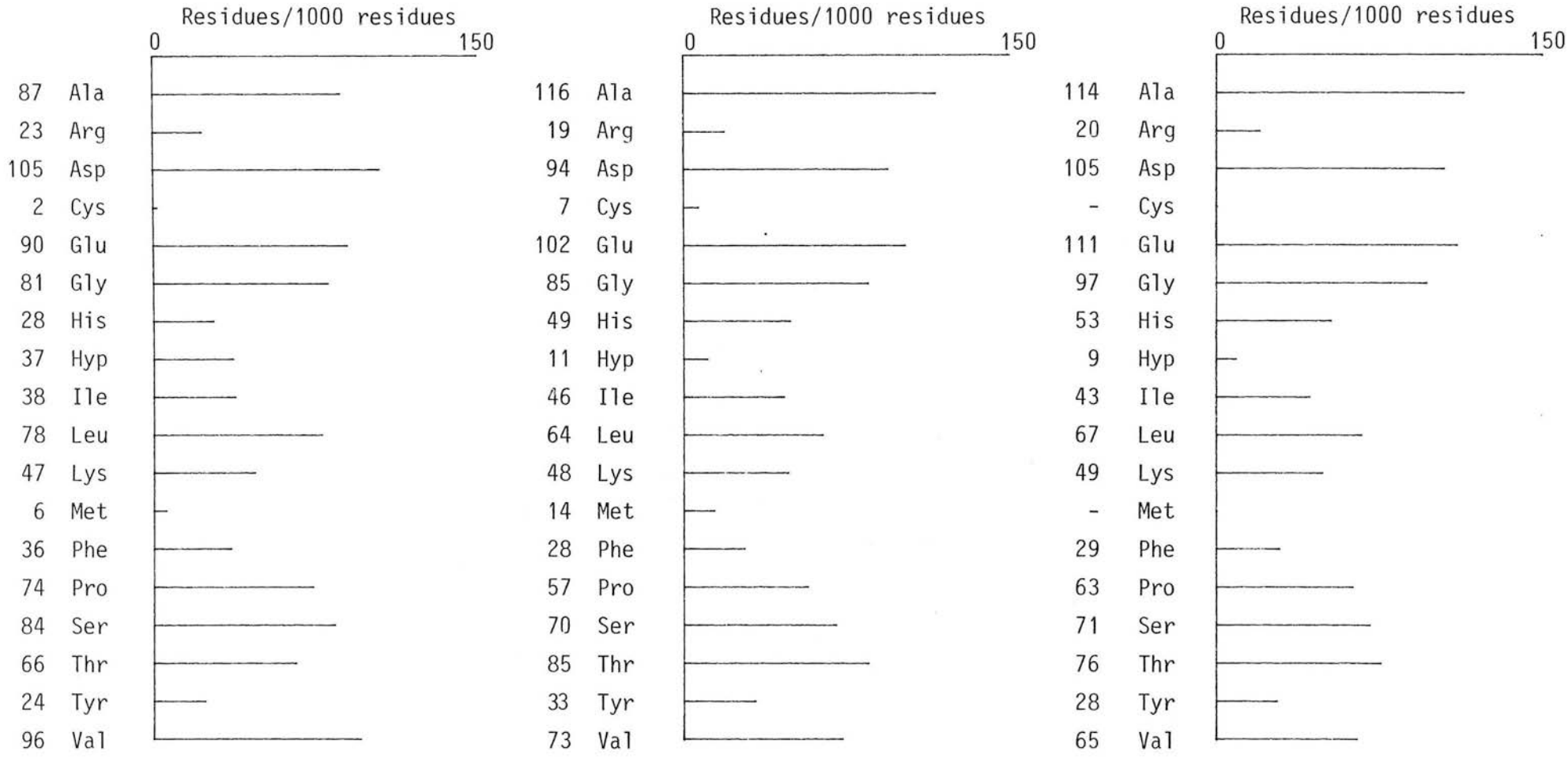
9.1%); nitrogen contents decreased from 3.17% and 4.10% to 2.22% and 2.23%; intrinsic viscosities were similar (330 $ml g^{-1}$ and 260 $ml g^{-1}$, 250 $ml g^{-1}$ and 250 $ml g^{-1}$); specific rotations increased from $+45^{o}$ and $+38^{o}$ to $+60^{o}$ and $+43^{o}$ respectively.

The similarities between the ash contents, intrinsic viscosities and specific rotations of the test faecal extracts and re-extracted gum karaya indicated the presence of gum karaya in faeces.

The results for the test faecal extracts were compared with those for gum karaya alone (Table VI.1). Ash contents were similar (0.0% and 9.7% and 1.1% and 9.1%); nitrogen contents increased from 0.08% and 0.00% to 2.22% and 2.23%; intrinsic viscosities decreased from 535 $ml g^{-1}$ and 515 $ml g^{-1}$ to 250 $ml g^{-1}$ and 250 $ml g^{-1}$; specific rotations were similar ($+59^{o}$ and $+43^{o}$, $+60^{o}$ and $+45^{o}$ respectively). The similarities between the ash contents and specific rotations of the test faecal extracts and gum karaya again indicated that gum karaya in an impure form was recovered from faeces after diet supplementation.

Gum karaya, the control faecal extract, and the test faecal extract from gum karaya diet supplementation were submitted to amino acid analysis. The amino acid profiles are shown in Figure VI. The three major amino acids in gum karaya were aspartic acid, valine and glutamic acid; in the control and test faecal extracts they were alanine, glutamic acid and aspartic acid. The major amino acids in the control and test faecal extracts were identical but the relative

FIGURE VI : Amino acid profiles



Major amino acids (i) Asp Val Glu (ii) Ala Glu Asp (iii) Ala Glu Asp
Footnote : key to amino acids in Figure IV.2. FD : freeze-dried.

proportions of glutamic acid and aspartic acid were increased in the test faecal extract. The proportions of arginine, aspartic acid, leucine, phenylalanine, proline and serine were higher in gum karaya and the test faecal extract than in the control faecal extract. Hence there are indications, from the amino acid analyses, that the proteinaceous component gum karaya appears in faeces from rats fed a diet supplemented with gum karaya. As the protein content of gum karaya is only ca. 0.5% it would be difficult to obtain a more positive conclusion because of the small quantity of gum karaya incorporated in the rat diet.

When faeces from rats on gum karaya diet supplementation were mixed with ammonium hydroxide solution the resultant mixtures were similar in appearance to that given by gum karaya in ammonium hydroxide. The test faecal mixture was very viscous. Faecal output from rats on gum karaya diet supplementation was increased (ca. 30%) compared with the faecal output from control rats.

Similarities between the ash contents and specific rotations of gum karaya alone and the test faecal extracts, and between the ash contents and intrinsic viscosities of re-extracted gum karaya and the test faecal extracts therefore indicate the presence of gum karaya or material similar to gum karaya in rat faeces after diet supplementation.

It has been postulated (15) that degradation of the exterior chains of gum karaya may take place in the rat gut. However, at-

tempts to measure molecular weights by the light scattering technique were unsuccessful because of problems associated with solubility and 'clean-up' of the faecal extracts. There were no indications of major degradation having taken place. It is possible that aggregation may have occurred due to the high emulsifying power of gum karaya.

The elemental diet, 'Flexical' was used in the second set of experiments.

Gum karaya was put through the extraction procedure and 74% was recovered for the reasons mentioned earlier (page73). When gum karaya was mixed with faeces from control rats and re-extracted, the yield was also 74%.

The analytical data for gum karaya and for re-extracted gum karaya are compared in Table VI.2.

Ash contents were similar (0.0% and 9.7% and 0.4% and 8.4%); nitrogen contents increased from 0.08% and 0.00% to 1.14% and 1.68%; intrinsic viscosities decreased from 535 $ml g^{-1}$ and 515 $ml g^{-1}$ to 400 $ml g^{-1}$ and 450 $ml g^{-1}$; specific rotations were similar ($+59^{o}$ and $+45^{o}$, $+60^{o}$ and $+53^{o}$ respectively).

The increased nitrogen contents of re-extracted gum karaya can only arise from the presence of contaminants from faeces. Molecular weights were not available for comparison because of the experimen-

tal difficulties found to be involved with solution clean-up.

When control faeces were extracted, the isolated material (total weight 0.3g) had a high nitrogen content (11.9%) and relatively low intrinsic viscosity (150 $ml g^{-1}$).

Gum karaya was incorporated into the elemental diet at a dose level of 1.2g/day/rat. The recovery of gum from faeces, based on the weight of gum karaya incorporated into the elemental foodstuff was 20%. As mentioned earlier (page 75) considerable mechanical losses were involved.

The analytical data for the test faecal extracts and re-extracted gum karaya are shown in Table VI.2. Ash contents were similar (0.4% and 8.4% and 0.0% and 9.1%); nitrogen contents decreased from 1.14% and 1.68% to 0.57% and 0.84%; intrinsic viscosities increased from 400 $ml g^{-1}$ and 450 $ml g^{-1}$ to 520 $ml g^{-1}$ and 530 $ml g^{-1}$; specific rotations decreased from $+60^{o}$ and $+53^{o}$ to $+58^{o}$ and $+41^{o}$ respectively.

The differences in the nitrogen contents, intrinsic viscosities and specific rotations between the test faecal extracts and re-extracted gum karaya were possibly a reflection of the differences in purity of the samples.

The analytical data for the test faecal extracts were also compared with those for gum karaya. Ash contents were similar (0.0%

TABLE VI.2 : Analytical data for gum karaya, gum karaya mixed with faeces and re-extracted, faecal extracts from (i) control rats and (ii) test rats given 1.2g gum karaya/rat/day. All rats fed the elemental diet.

	GUM KARAYA		FAECAL EXTRACTS (Gum karaya mixed with faeces)		CONTROL FAECAL EXTRACTS		FAECAL EXTRACTS (Gum karaya at 1.2g/rat/day)	
Total weight of extracts (g)			7.4		0.3		7.2	
Total yield, %	74		74				20	
	AP	FD	AP	FD	AP	FD	AP	FD
Moisture, %	12.5	15.3	9.8	11.8	nd[+]	10.3[*]	13.3	11.6
Ash, %[a]	0.0	9.7	0.4	8.4	"	nd[+]	0.0	9.1
Nitrogen, %[a]	0.08	0.00	1.14	1.68	"	11.9	0.57	0.84
Intrinsic viscosity[a,c] ml g^{-1}	535	515	400	450	"	154	520	530
Specific rotation,[a,b] degrees	+59	+45	+60	+53	"	nd[+]	+58	+41
Molecular weight,[a,c] $\bar{M}w \times 10^6$	4.6	4.7	nd[++]	nd[++]	"	nd[+]	4.6	4.9

Footnotes : AP : precipitated with 4 volumes ethanol containing 1% (v/v) concentrated HCl
FD : freeze-dried
*estimated
nd[+] : not done due to small quantity of sample
nd[++] : see page 79
a : corrected for moisture content
b : in 0.2M ammonium hydroxide
c : in 0.1M ammonium hydroxide and 1.0M sodium chloride

and 9.7% and 0.0% and 9.1%); nitrogen contents increased from 0.08% and 0.00% to 0.57% and 0.84%; intrinsic viscosities, specific rotations and molecular weights were all similar, with values of 535 mlg^{-1}, 515 mlg^{-1}, 520 mlg^{-1} and 530 mlg^{-1}; $+59^{o}$, $+45^{o}$, $+58^{o}$ and $+41^{o}$; 4.6×10^{6}, 4.7×10^{6}, 4.6×10^{6} and 4.9×10^{6} respectively.

Ash contents, intrinsic viscosities, specific rotations and molecular weights for the test faecal extracts correlated well with those for gum karaya, indicating the presence of gum karaya in the faecal extracts.

As with the standard diet, faecal output increased (ca. 30%) when the elemental diet was supplemented with gum karaya. When faeces from rats given gum karaya were mixed with ammonium hydroxide solution, the solution viscosity closely resembled that of gum karaya in ammonium hydroxide. The faecal mixture was very viscous.

There is therefore no clear evidence as to whether gum karaya is or is not degraded on ingestion by the rat when included in a standard or elemental diet. It is however, apparent that substances can be extracted from (a) test faeces (standard diet) with ash contents, intrinsic viscosities and specific rotations similar to those for re-extracted gum karaya and (b) test faeces (elemental diet) with ash contents, intrinsic viscosities, specific rotations and molecular weights very similar to those for gum karaya.

It was found to be very difficult to extract gum karaya quan-

titatively from rat faeces. It is possible that gum karaya, an efficient emulsifier, emulsifies proteinaceous and fatty substances with which it comes into contact on passage through the rats digestive system; if so, gum karaya complexed with such substances would not be freely extractable as gum karaya.

It was possible to demonstrate that when the elemental diet was supplemented with gum karaya a high molecular weight and highly viscous substance similar to gum karaya was extracted from faeces. However, no molecular weight values could be obtained for test faecal extracts from experiments involving the standard diet. In the standard diet, gum karaya is incorporated into a diet containing vitamins, crude protein, crude fat, mineral matter, starch, digestible carbohydrates and crude fibre, some of which will travel with gum karaya through the alimentary and digestive tracts. The elemental diet consists of carbohydrate, protein and fat in a form that does not require intact digestive capabilities; its components are totally digested in the upper intestinal tract.

The indications that gum karaya is not degraded to any great extent when ingested by the rat supports the prior evidence of Elsenhans et al. 913) and by Brown et al. (15) that gum karaya is an inert polysaccharide, undegraded by intestinal bacteria. Salyers et al. (20, 21) have established that, of all the strains of Bacteroides surveyed in the human colon, none utilised gum karaya.

VI.5 CONCLUSIONS

1. Faecal extracts obtained from rats fed the standard diet supplemented with gum karaya at 1.2g/day/rat were shown to be similar but not identical to re-extracted gum karaya (mixed with faeces from control rats and re-extracted).

2. Faecal extracts obtained from rats fed the elemental diet supplemented with gum karaya at 1.2g/day/rat were shown to be similar but not identical to gum karaya.

3. It was not possible to determine whether the gum karaya extracted from test faeces was degraded or not, particularly through the difficulties found to be associated with molecular weight measurements of the impure forms of the gum that were extracted. There are analytical indications that gum karaya is not degraded extensively within the rat but it is not possible, from these experiments, to conclude that the original gum karaya macromolecules were completely undegraded.

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SECTION VII

ANALYTICAL STUDIES OF SAMPLES OF GUM TRAGACANTH (GENUS *ASTRAGALUS*) OF IRANIAN AND TURKISH ORIGIN

VII.1 INTRODUCTION

VII.1(a) Gum tragacanth (Astragalus spp.)

Astragalus, the largest genus of the Angiosperms (flowering plants) is also the largest genus (1600 species) within the Family Leguminosae, to which gum arabic belongs (1,2). Astragalus species (1) are found throughout Turkey (1), the Sahara (3), China (4), Afghanistan (5), Africa, Japan, Syria (6), Iran (7), North-West Himalayas, West Pakistan (8), Egypt (9), Argentina, Bolivia and Chile (10).

The genus Astragalus can be divided botanically into sub-series according to whether the species are annual or perennial, shrubs or herbs and according to the type of leaves they possess. These sub-series can be further divided into different sections, which may contain from one to over sixty species. These sub-series and sections are detailed in Table VII.1. Of the 1600 known Astragalus species, very few are gum-bearing. Three well-known species produce gum; Astragalus gummifer Labillardière, Astragalus microcephalus Willdenow and Astragalus kurdicus Boissier. Astragalus gummifer is found (Table VII.1) in sub-series Tragacantha, section Platonychium; Astragalus microcephalus and Astragalus kurdicus occur in sub-series Tragacantha, section Rhacophorus (1,6,7, 11).

Astragalus microcephalus, and not the traditionally accepted Astragalus gummifer, has been confirmed as the major source of good

TABLE VII.1 : Sub-series of Astragalus (1, 6, 7, 11)

(1) EPIGLOTTIS

Eupiglottis
Herpocaulos

(2) TRIMENIAEUS

Epiglottis
Oxyglottis
Pentaglottis
Harpilobus
Ankylotus
Cyamodes
Aulacolobus
Buceras
Platyglottis
Falcinellus
Cycloglottis
Drepanodes

(3) HYPOGLOTTIS

Dasphyllium
Hypoglottis
Stereothrix
Malacothrix
Brachylobium
Tapinodes
Hermiphragmium
Euhypoglottis
Heterozyx

(4) PHACA

Glycophyllos
Galegiformis
Theiochrus
Christiana
Erionotus
Macrosemium
Myobroma
Chronopus
Acanthopace
Coluteocarpus
Aegacantha
Pseudoastereothrix

(5) TRAGACANTHA

Brachycalyx
Platonychium
Adiaspastus
Diacme
Rhacophorus
Macrophyllium
Pterophorus
Stenonychyium

(6) CALYCOPHYSA

Hymenostegis
Poterium
Megalocystis
Halicacabus
Stereocalyx
Hymenocoleus
Rhabdotus
Argaeus
Alopecias
Grammocalyx
Tricholobus
Campylanthus
Eremophysa

(7) CERCIDOTHRIX

Euodmus
Craccina
Ornithopodium
Hololeuce
Acmothrix
Sisyrophorus
Trachycercis
Proselius
Xiphidium
Ammondendrum
Melanocercis
Cystium
Ereoceras
Tamias
Cremoceras
Leucocercis
Caraganella

(8) CALYCOCYSTIS

Eustales
Vulneraria
Sphaerocystis
Cystodes
Cysticalyx
Laguropsis

quality gum tragacanth in Central and South-East Anatolia (12). Poorer quality gum tragacanth, yielded by Astragalus gummifer and Astragalus kurdicus, is termed 'Traganton' for some commercial purposes (13, 14).

The name 'tragacanth', derived from the Greek tragos (goat) and akantha (horn), probably refers to the best grade of gum which is exuded in long, flat, flexible, almost opaque ribbons. The gum also occurs in a flake form which tends to be oval, dark and brittle (15).

Gum tragacanth is a highly valued commercial gum exuded from the stems and roots of Astragalus shrubs after tapping i.e. incisions made with a special knife at the start of the hot, dry season. As the gum exudes, water is absorbed from the surrounding tissues, the gum eventually hardens and seals the incision. The fact that gum tragacanth is produced immediately after incisions are made leads to the view (12) that the gum precursors are already present within the plant. The gum also exudes from plants damaged by the wind or by grazing animals, but the best quality gum is obtained in response to tapping. Rapid drying in calm, hot, dry weather gives almost colourless good grade gum; dust storms and rain contaminate the exuding gum with soil and sand and such samples are inferior. Young shrubs (1-5 years old) give best quality gum (12, 15).

Gum tragacanth is a complex polysaccharide associated with magnesium, calcium and potassium salts, proteinaceous material, cel-

lulose and/or starch. The gum has a methoxyl content of ca. 3% (16,17). A 1% (w/v) aqueous solution of gum tragacanth is slightly acidic (pH 5-6) (16, 18, 19). On acidic hydrolysis, the gum yields D -galacturonic acid, D-galactose, L -arabinose, D-xylose, L -fucose and L-rhamnose (20).

Gum tragacanth is a very variable, complex substance. The flow times of aqueous solutions of commercial samples of gum tragacanth varied (21) in a standardised flow-time test, from <1 second to 1700 seconds; only four samples had flow times greater than 100 seconds.

Gum tragacanth is not completely water-soluble. It is possible to separate a water-insoluble portion (bassorin) from a water-soluble portion (tragacanthin). The relative proportions of the water-insoluble and water-soluble components have been reported to be 70%:30% (16,22), 60%:40% (23) and 50%:50% (24).

There have been diverse reports concerning the polysaccharide components of gum tragacanth. Selby (24) stated that the methoxyl content of the gum was mainly associated with tragacanthin; Fellenberg (25) reported that there was no methoxyl content in tragacanthin. Later, it was reported (26) that methoxyl groups were associated with both bassorin and tragacanthin.

James and Smith (17, 27) were able to fractionate gum tragacanth into three components; tragacanthic acid, a neutral polysac-

charide and a glycoside, the neutral polysaccharide being an arabinogalactan.

Aspinall and Baillie (23) studied a commercial sample of gum tragacanth; after separating the gum into its two components, the tragacanthin was fractionated further into tragacanthic acid and an arabinogalactan. Tragacanthic acid which yielded D-galacturonic acid, D-xylose, L-fucose and D-galactose on acid hydrolysis, consisted of linear chains of α-1, 4- linked D-galacturonic acid residues, the majority of which carried D-xylose-containing side-chains linked through carbon 3. Three types of side-chains were identified: single β- D-xylopyranose residues; disaccharide units of 2-O--α-L-fucopyranosyl- D-xylopyranose and 2-O-β- D-galactopyranosyl- D-xylopyranose. On acid hyrolysis, the arabinogalactan yielded L-arabinose, D-galactose, D-galacturonic acid and traces of L-rhamnose; interior chains of D-galactose residues were found to be surrounded by L-arabinose residues. Fractional precipitation of tragacanthin, by addition of ethanol, led to polysaccharide fractions which differed markedly in optical rotation, equivalent weight and sugar composition. Some of these fractions appeared to consist largely of either tragacanthic acid or arabinogalactan.

The water-insoluble portion (bassorin) of gum tragacanth which dissolved partially in dilute aqueous ammonium hydroxide, remained in solution after acidification. Graded precipitation with ethanol gave fractions similar to those isolated from the water-soluble component. The alkali-insoluble component appeared to be a glucan

with adhering tragacanthic acid and arabinogalactan.

VII.1(b) Properties and uses of gum tragacanth

Gum trayacanth is widely used as a natural emulsifier and thickener in the food, drug and allied industries. The food industry is the major market for gum tragacanth hence it is essential that the gum has no harmful effects when ingested. The gum, accepted within the USA since 1961 as 'Generally Recognised as Safe' (GRAS) at the 0.2 - 1.3% level in foodstuffs (28,29) is used extensively in salad dressings, relishes, sauces, condiment bases, sweet pickle liquors, soft-jellied products, thick broths, beverage and bakery emulsions, ices and sherbets bakery toppings and fillings and confectionery cream centres (15).

Gum tragacanth dispersions have a soothing and lubricating action in medications such as ointments for burns (30).

The high viscosity of gum tragacanth in water at low concentration makes it useful for preparing aqueous suspensions of insoluble substances for toxicity studies of orally administered products such as phenacetin (31), kaolin (32), barium sulphate (33) and paracetamol (34).

VII.1(c) A short review of dietary studies of gum tragacanth in animals and in Man

Gum tragacanth has been reported (35, 36) to inhibit the growth of ascites tumour cells in mice. The better grade samples were found to have a greater inhibitory effect, although the effect was present in all grades.

Vohra and Kratzer (37) concluded that gum tragacanth, fed to chickens in a nutritionally-balanced diet at a level of 2% (w/w), caused a depression in their growth. But when gum tragacanth was incorporated into the diet of cockerels at 3% (w/w), toxic effects were not observed (38).

A 1% (w/v) solution of Iranian gum tragacanth caused the death of all foetuses when administered to pregnant mice, between the 11th and 21st day of gestation, as peritoneal injections but not when administered orally or subcutaneously. The fetotoxic effect of the gum was ascribed to the metabolic products from enterobacteria present in the sample, with a direct effect on the uterus and its vascular system (39).

Boyd (40) reported that gum tragacanth, fed to albino rats at concentrations of 2% (w/v) in dosages of 2g/kg every 90 minutes to give final dose levels of 8, 9, 10 and 12g/kg/day by oral or intragastric routes, caused lethal and toxic reactions. An initial toxic reaction, during the first two days at all dose levels, was followed

by adaptation and development of tolerance in some cases. The toxic symptoms included dyspnoea, pallor, convulsions, marked loss of body weight, hypothermia, glycosuria, aciduria and finally death. Autopsy revealed local inflammation of the heart and stomach and congestion and degeneration of many organs. Control animals did not show adverse reactions to the large amounts of water given.

The dose levels of gum tragacanth in the above study were very high in comparison to the quantities used in foodstuffs.

Elsenhans et al. (41) investigated the effect of gum tragacanth on rat intestinal transport of monosaccharides and neutral amino acids in vitro using rings of rat small intestine. The substances used were α-methyl- D-glucoside, D-galactose, L-leucine and L-phenylalanine. Gum tragacanth was found to affect their uptake as it raised the viscosity of the medium, creating a physical barrier in the form of an unstirred layer. The process could be reversed by washing the tissue free of polysaccharide or by increasing the agitation of the mixture. Whole gum tragacanth was not used in this study; the water-insoluble component, bassorin, had been removed to leave only the water-soluble component, tragacanthin.

Strobel et al. (42) investigated the immune response of gum tragacanth in mice; the gum elicited a response comparable to that produced by hen's egg albumin.

Transmission electron microscopy was used (43) to examine the

ultrastructure of rat hearts and livers after gum tragacanth had been included in the diet at 0.05%, 1.5%, 2.5% and 3.5% (w/w) for 91 days. Abnormalities were not detected in any of the organelles in the heart and liver specimens from any of the test animals. No pathological changes were observed; all micrographs showed normal healthy tissues. Data from assays of the microsomal protein and cytochrome P450 contents of the livers showed that the gum did not cause any inductive effects (43). These results therefore did not support the suggestions of Bachmann and Zbinden (44) and Bachman et al. (45) that gum tragacanth causes inductive changes in the function of rat heart and liver mitochondria. However, they do confirm a report by Zbinden (46) that ingestion of gum tragacanth does not produce abnormalities in the cardiac function of rats.

In 1984, a dietary study of gum tragacanth in human volunteers was reported (47). After a control period of 7 days, 5 male volunteers incorporated 9.9g gum tragacanth daily into their diet for 21 days. Measurements made before and after the test period showed that ingestion of gum tragacanth had no significant effect on the following: plasma biochemistry, haematological indices, urinalysis parameters, glucose tolerance, serum cholesterol, triglycerides, phospholipids, breath hydrogen and breath methane concentrations. The intestinal transit time increased and faecal fat concentrations increased in 4 of the 5 subjects. Faecal wet and dry weights increased in all subjects. No adverse effects were observed. The daily intake was very high in relation to the small amounts normally used in foodstuffs; on the basis of UK and EEC im-

port statistics, the amount ingested in foodstuffs can be estimated at only ca 2g per person per annum within the U.K. if the amounts imported are consumed equally by all members of the population.

VII.1(d) Objectives

The aims of the studies undertaken were: (a) to determine and compare the physico-chemical parameters of several commercial samples of gum tragacanth of Iranian and Turkish origin and to investigate the nature of the differences between them; (b) to attempt to clarify conflicting reports e.g. concerning the ratio of the water-insoluble to water-soluble components and (c) to characterise the sample of gum tragacanth used as the Test Article in the dietary study in Man (47).

VII.2 METHODS AND MATERIALS

VII.2(a) Origin of samples

Commercial samples of food grade gum tragacanth of Iranian origin were supplied by major importers in West Germany (samples 1,2,3 and 7) and in Great Britain (samples 4,5 and 6). Commercial samples of gum tragacanth of Turkish origin (samples 8-12) were supplied by a major Turkish exporter, who also supplied a sample of 'Traganton'.

The samples of gum tragacanth from Astragalus gummifer Labillardière and Astragalus microcephalus Willdenow were collected at Hizan in the Bitlis province of East Anatolia by Dr. M. Dogan and Dr. T. Ekim in August 1984. Reference vouchers for these samples are lodged at Ankara University Herbarium (ANKA). The sample of gum tragacanth from Astragalus kurdicus Boissier was provided by Mr. S. Taranto of Istanbul.

VII.2(b) Analytical methods

Moisture, ash and methoxyl contents, intrinsic viscosity, and molecular weight determinations and amino acid analyses were carried out as detailed in Section II of this Thesis.

Nitrogen contents were determined by the Chemistry Department, St. Andrews University, with a Carlo-Erba Autoanalyser.

Viscosity determinations were made with a Brookfield Synchro-electric viscometer, Model RVF (Brookfield Engineering Laboratories, Stoughton, Massachusetts). Solutions (1%, w/v) were prepared by wetting the gum sample with drops of ethanol, adding distilled water and allowing hydration to take place over 24 hours. Brookfield spindle 3 was used at 20 rpm in all determinations at 20^{o}C.

Analyses of sugars. Samples (ca. 200 mg) were hydrated overnight then made 0.5M with respect to sulphuric acid and hydrolysed overnight in a boiling water bath. The hydrolysate was treated as

detailed in Section II of this Thesis. For paper chromatographic separations, the eluant was ethanol, hydrochloric acid (0.1M), butanol (10 : 5 : 1, v/v).

VII.2(c) Separation of gum tragacanth into water-insoluble and water-soluble components

Gum tragacanth (6g) was dispersed in distilled water (900 ml) and stirred gently overnight. The mixture was centrifuged (Sorvall RC-SB refrigerated Superspeed centrifuge) for 35 minutes at 10,000 rpm. The water-soluble supernatant was decanted off. The water-insoluble component was left overnight in distilled water; the mixture was centrifuged as before and the water-soluble supernatant was decanted and added to the first extract. The water-soluble component was concentrated by rotary evaporation. Both components were freeze-dried.

VII.3 RESULTS

Table VII.2 presents analytical data for seven commercial samples of gum tragacanth from Iran. Table VII.3 presents analytical data for six commercial samples of gum tragacanth from Turkey. Table VII.4 presents analytical data for gum tragacanth samples from *Astragalus kurdicus*, *Astragalus gummifer* and *Astragalus microcephalus* and for a sample of 'Traganton' from Turkey. Tables VII.5, VII.6 and VII.7 present analytical data for the water-insoluble and

water-soluble components of samples 1, 3, 4, 5, 6 and 7 from Iran and for samples 8, 9, 10, 11, 12, 'Traganton' and a sample from *Astragalus kurdicus*, from Turkey.

Figures VII.2 to VII.11 detail amino acid profiles for several of the whole gum tragacanth samples and for some of their water-insoluble and water-soluble components. Figure VII.2 also presents the amino acid profile for a sample of gum arabic (*Acacia senegal*).

VII.4 DISCUSSION

VII.4(1) Gum tragacanth samples from Iran

Analytical data for seven gum tragacanth samples from Iran are detailed in Table VII.2. Moisture contents were found to vary from 10.3% to 12.6%; ash contents from 3.3% to 4.1%; protein contents from 0.50% to 3.25% and Brookfield viscosities from 260 centipoise to 3385 centipoise.

All samples gave the following sugars on acidic hydrolysis; D-galacturonic acid, D-galactose, L-arabinose, D-xylose, L-fucose, L-rhamnose and traces (<1%) of D-glucuronic acid and D-glucose. There were however variations in the sugar ratios given by the different samples. The predominant sugars in samples 1 and 2 were L-fucose and D-xylose; in samples 3,4,5,6 and 7 the major sugar components were L-arabinose and D-xylose.

TABLE VII.2 : Analytical data for commercial samples of gum tragacanth (Astragalus spp.) from Iran.

	1	2	3	4	5	6	7	Average of 1-7
Moisture, %	11.8	12.6	12.0	12.3	12.5	10.3	11.3	11.8
Ash, %[a]	3.7	3.8	3.6	3.5	4.1	3.4	3.3	3.6
Nitrogen, %[a]	0.08	0.10	0.40	0.33	0.27	0.51	0.52	0.30
Protein, %[a]	0.50	0.63	2.50	2.06	1.69	3.19	3.25	1.97
Methoxyl, %[b]	4.8	4.6	4.0	3.6	3.8	2.7	2.8	3.7
Viscosity (centipoises)	3385	3040	1480	1080	260	425	430	1440
After hydrolysis:-								
Galacturonic acid, %	18	16	16	13	11	10	9	13
Galactose, %	5	6	8	12	12	13	14	10
Arabinose, %	10	16	27	32	36	45	49	30
Xylose, %	29	28	25	21	20	18	17	23
Fucose, %	34	30	20	18	18	10	8	20
Rhamnose, %	4	4	4	4	3	4	3	4
Ratio, insoluble to soluble components	65:35	60:40	50:50	60:40	50:50	50:50	40:60	

Footnotes : a : corrected for moisture content b : corrected for moisture and protein content

The ratios of the water-insoluble to water-soluble components varied from 40:60 to 65:35.

The seven samples studied represented different commercial grades of gum tragacanth; for commercial purposes, viscosity and colour are the most important determinations of quality. The gum samples studied show variation in protein, methoxyl and sugar contents, in Brookfield viscosity and in the ratios of the water-insoluble to water-soluble components. The best quality sample 1, was found to have the highest methoxyl, L-fucose, D-xylose and D-galacturonic acid contents, the highest Brookfield viscosity, the highest ratio of water-insoluble to water-soluble component and the lowest protein content.

The nitrogen content of the gum tragacanth samples was shown to be proteinaceous; the amino acid profiles are detailed in Figures VII.2 to VII.6. In all cases, the prominent amino acids were hydroxyproline, proline, serine and valine, plus aspartic acid in samples 1, 5 and 7 and histidine in samples 3, 4 and 6. The amino acid profiles for the Iranian gum tragacanth samples were similar to that for gum arabic (Figure VII.2) in which the prominent amino acids are hydroxyproline, serine, proline, leucine and aspartic acid. Gum arabic (*Acacia senegal*) is also derived from trees belonging to the Family *Leguminosae*.

VII.4(2) Gum tragacanth samples from Turkey

Analytical data for gum tragacanth samples from Turkey are detailed in Table VII.3. Moisture contents were found to vary from 10.6% to 12.8%; ash contents from 3.5% to 7.1%; protein contents from 2.13% to 3.31%; methoxyl contents from 2.6% to 4.0% and Brookfield viscosities from 40 centipoise to 540 centipoise.

All samples yielded the following sugars on acid hydrolysis; D-galacturonic acid, L-galactose, L-arabinose, D-xylose, L-fucose, L-rhamnose with traces (<1%) of D-glucuronic acid and D-glucose. The predominant sugars were L-arabinose and D-xylose in samples 8, 9 and 10 and L-arabinose and D-galactose in samples 11 and 12. The ratio of the water-insoluble to water-soluble component was 50:50 in all five samples. The Brookfield viscosities of the Turkish gum tragacanth samples were found to be generally lower than those of the Iranian gum tragacanth samples.

The amino acid profiles for the Turkish samples 8-12 are detailed in Figures VII.7, VII.8 and VII.9. As for the Iranian samples, in all cases, the most prominent amino acids were hydroxyproline, serine, proline and valine, plus histidine in samples 8, 9, 10 and 11 and aspartic acid in sample 12. These results correlate well with those for the Iranian samples, demonstrating that the protein content of the twelve samples were similar, althougn their sugar compositions varied extensively.

TABLE VII.3 : Analytical data for commercial samples of gum tragacanth (Astragalus spp.) from Turkey

	8	9	10	11	12	Average of 8-12
Moisture, %	11.4	11.6	10.6	13.1	12.8	11.9
Ash, %	3.5	3.9	4.0	7.1	4.5	4.6
Nitrogen, %[a]	0.34	0.36	0.44	0.36	0.53	0.41
Protein, %[a]	2.13	2.25	2.75	2.25	3.31	2.54
Methoxyl, %[b]	4.0	3.7	3.5	3.5	2.6	3.5
Viscosity (centipoises)	540	145	80	60	40	170
After hydrolysis:-						
Galacturonic acid, %	15	15	13	14	9	13
Galactose, %	14	16	14	19	17	16
Arabinose, %	33	35	34	38	42	36
Xylose, %	22	17	21	18	15	19
Fucose, %	12	11	13	9	10	11
Rhamnose, %	4	6	5	2	7	5
Ratio, insoluble to soluble components	50:50	50:50	50:50	50:50	50:50	

Footnotes : a : corrected for moisture content b : corrected for moisture and protein content

Analytical data for 'Trayanton' and the gum tragacanth samples from Astragalus kurdicus and Astragalus gummifer from Turkey are detailed in Table VII.4. Moisture contents were found to vary from 9.9% to 13.1%; ash contents from 2.0% to 2.9%; protein contents from 2.50% to 2.88%; methoxyl contents from 0.7% to 0.9% and the ratios of their water-insoluble to water-soluble components ranged from 60:40 to 70:30. Analytically, these three samples are very similar; their predominant sugars were L -arabinose and D -galactose. These samples can therefore be regarded as arabinogalactans with traces of D-galacturonic acid, D-xylose, L-fucose and L-rhamnose.

Macromolecules containing L-arabinose and D-galactose have been found in many plant tissues. In some situations they have been isolated as polysaccharides free from associated protein; in other cases they occur in covalent association with protein (48). Gum arabic (Acacia senegal) which, like gum tragacanth (Astragalus spp.) belongs to the family Leguminosae, has been termed an arabinogalactan-protein (49).

The amino acid profiles for 'Traganton' and the samples from Astragalus gummifer and Astragalus kurdicus are detailed in Figures VII.10 and VII.11. In all three samples, the most prominent amino acids were hydroxyproline, aspartic acid, serine and valine plus alanine in 'Trayanton', leucine in the sample from Astragalus gummifer, and histidine in the sample from Astragalus kurdicus. There was good correlation between the amino acid profiles of these samples.

TABLE VII.4 : Analytical data for 'Traganton' and samples of gum tragacanth from Astragalus kurdicus, Astragalus gummifer and Astragalus microcephalus.

	'Traganton'	Astragalus kurdicus	Astragalus gummifer	Astragalus microcephalus
Moisture, %	10.6	13.1	9.9	12.7
Ash, %[a]	2.8	2.0	2.9	3.2
Nitrogen, %[a]	0.40	0.46	0.46	0.58
Protein, %[a]	2.50	2.88	2.84	3.65
Methoxyl, %[b]	0.7	0.7	0.9	3.3
After hydrolysis:-				
Galacturonic acid, %	3	2	3	11
Galactose, %	29	28	23	14
Arabinose, %	56	63	63	37
Xylose, %	5	3	5	22
Fucose, %	2	Trace	2	12
Rhamnose, %	5	4	4	4
Ratio, insoluble to soluble components	70:30	70:30	60:40	35:65

Footnotes : a : corrected for moisture content b : corrected for moisture and protein content

It has been reported that 'Traganton' is derived from Astragalus kurdicus and/or from Astragalus gummifer (13, 14) and that it is a low quality variant of true gum tragacanth. It has been established here, that gum 'Traganton' and the gums from Astragalus kurdicus and Astragalus gummifer have similar sugar and amino acid compositions.

Analytical data for the Turkish sample of gum tragacanth from Astragalus microcephalus are detailed in Table VII.4. The moisture content was 12.7%; ash content, 3.2%; protein content, 3.65%; methoxyl content, 3.3% and the ratio for the water-insoluble to water-soluble components was 35:65. The sugars yielded on acid hydrolysis were D -galacturonic acid; D -galactose; L -arabinose; D -xylose; L -fucose and L -rhamnose with traces (<1%) of D -glucuronic acid and D -glucose. The most predominant sugars were L -arabinose and D -xylose; this is comparable to samples 3,4,5,6 and 7 from Iran and samples 8,9 and 10 from Turkey.

The amino acid profile for the Turkish sample from Astragalus microcephalus (Figure VII.11) was similar to the gum tragacanth samples 1-7 from Iran and samples 8-12 from Turkey.

These results substantiate the fact that Astragalus microcephalus is now accepted as the major source of Turkish commercial gum tragacanth (12).

Hydroxyproline was the most prominent amino acid in all the

gum tragacanth samples studied. At one time, hydroxyproline was considered to be a constituent of only animal proteins, but its presence in plants is becoming increasingly apparent. Hydroxyproline-containing glycoproteins are important constituents of plant cells having a structural role in the primary cell wall.

Very high proportions of hydroxyproline are also found in gum arabic (Acacia senegal) (49,51) and in the gums from Prosopis spp. (52). The genera Astragalus, Acacia and Prosopis are members of the Family Leguminosae; gums from genera in other Families e.g. Sterculia (53) do not contain high proportions of hydroxyproline. In the animal connective tissue proteins, collagen and elastin, hydroxyproline plays a unique role in stabilising their helical structures (54-56).

It has been demonstrated (23) that gum tragacanth is a complex, heterogeneous substance. It is now evident that gum tragacanth samples can differ markedly in their protein, methoxyl and sugar contents, viscosity and in the ratio of their water-insoluble to water-soluble components. The differing values for the ratio of the water-insoluble to water-soluble components reported in earlier studies fall within the range of values found here for the different samples investigated. The samples studied by other workers (21, 24-26) all differed and are now seen to have represented the different botanical species involved. All earlier studies were deficient in the respect that inadequate, if any, details were provided regarding either the botanical or the geographical source of the gum

tragacanth sample studied. Furthermore an explanation is now available for the diversity of quality that is well-known to occur commercially, between different samples of gum tragacanth, particularly between samples of Iranian and Turkish origin. Different Astragalus species are involved and each species gives an exudate of different composition.

VII.4(3) The Test Article used in a dietary study of gum tragacanth in Man

As not more than ca. 10% of the world's supply of foodstuffs grade gum tragacanth is exported from Turkey an Iranian sample of average quality was regarded as being the most representative sample of foodstuffs quality gum for the purposes of a dietary study in Man.

The Test Article ingested by volunteers was the Iranian gum tragacanth sample 4 (Table VII.2). When the analytical data for sample 4 are compared with those for the Iranian samples 1-7 (Table VII.2) it is clearly indicated that gum tragacanth sample 4 has properties and composition that are intermediate between all the Iranian samples studied. Thus, it is reasonable to claim that the Test Article selected was a gum tragacanth sample of fair average quality.

VII.4(4) Analysis of the water-insoluble and water-soluble components of gum tragacanth samples from Iran and Turkey

Analytical data for the water-insoluble and water-soluble components of gum tragacanth samples 1, 3, 4 and 7 from Iran are detailed in Table VII.5; for samples 5 and 6 from Iran and samples 8 and 9 from Turkey in Table VII.6 and for samples 10, 11, 12, 'Traganton' and from Astragalus kurdicus from Turkey in Table VII.7.

The water-insoluble components were found to have lower ash contents; higher protein contents and lower methoxyl contents (apart from sample 1) than the water-soluble components.

It was reported by Selby (24) that the methoxyl groups in gum tragacanth were mainly associated with the water-soluble component. Fellenberg (25) stated that there was no methoxyl content associated with the water-soluble component whereas Jannot and Gonnard (26) claimed that both components contained methoxyl groups. This study has confirmed that methoxyl groups are associated with both the water-insoluble and water-soluble components.

The intrinsic viscosities of the water-soluble components varied in relation to the Brookfield viscosities of the whole gum samples. Gum tragacanth samples of high Brookfield viscosity gave water-soluble components of high intrinsic viscosity. Attempts were made to solubilise the water-insoluble component of gum tragacanth using: heat; acid; alkali; sodium borohydride in both aqueous and

TABLE VII.5 : Analytical data for the water-insoluble and water-soluble components of gum tragacanth (*Astragalus* spp.) samples 1, 3, 4 and 7 from Iran

	1		3		4		7	
%	Insoluble 65	Soluble 35	Insoluble 50	Soluble 50	Insoluble 60	Soluble 40	Insoluble 40	Soluble 60
Moisture, %	11.3	11.5	10.3	11.1	10.6	10.8	8.0	8.0
Ash, %[a]	3.7	4.1	3.2	3.8	3.2	4.0	2.5	3.8
Nitrogen, %[a]	0.10	0.07	0.58	0.26	0.49	0.13	0.71	0.37
Protein, %[a]	0.63	0.44	3.63	1.63	3.08	0.81	4.44	2.31
Methoxyl, %[b]	5.1	4.5	3.8	4.1	3.6	4.3	2.5	3.0
Intrinsic viscosity[a] ml g^{-1}	np	1050	np	1070	np	1045	np	720
After hydrolysis:-								
Galacturonic acid, %	20	17	13	17	10	14	9	9
Galactose, %	5	7	8	10	11	12	15	12
Arabinose, %	12	14	26	28	35	31	41	44
Xylose, %	29	30	27	24	23	27	19	20
Fucose, %	31	28	22	17	18	14	13	10
Rhamnose, %	3	4	4	4	3	2	3	5

Footnotes : a : corrected for moisture content
b : corrected for moisture and protein content
c : not possible; no effective solvent available

TABLE VII.6 : Analytical data for the water-insoluble and water-soluble components of gum tragacanth (*Astragalus* spp.) samples 5 and 6 from Iran and 8 and 9 from Turkey.

	5		6		8		9	
%	Insoluble 50	Soluble 50	Insoluble 50	Soluble 50	Insoluble 50	Soluble 50	Insoluble 50	Soluble 50
Moisture, %	8.5	7.5	9.8	9.8	10.1	10.3	10.5	11.0
Ash, %[a]	3.6	4.4	2.6	3.8	3.0	3.8	3.5	4.3
Nitrogen, %[a]	0.31	0.25	0.72	0.33	0.40	0.25	0.53	0.16
Protein, %[a]	1.94	1.56	4.50	2.08	2.50	1.56	3.31	1.00
Methoxyl, %[b]	3.4	3.8	2.7	2.7	3.5	4.2	3.3	4.1
Intrinsic viscosity, ml g^{-1}	np	745	np	775	np	880	np	780

Footnotes : a : corrected for moisture content
b : corrected for moisture and protein content
np : not possible; no effective solvent available

TABLE VII.7 : Analytical data for the water-insoluble and water-soluble components of gum tragacanth (*Astragalus* spp.) samples 10, 11 and 12, 'Traganton' and *Astragalus kurdicus* from Turkey.

	10		11		12		'Traganton'		*Astragalus kurdicus*	
	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Soluble
%	50	50	50	50	50	50	70	30	70	30
Moisture, %	10.5	10.1	8.7	8.8	8.8	8.7	8.5	7.5	6.9	7.4
Ash, %	3.5	4.5	8.2	5.1	4.3	5.2	2.1	3.9	1.6	3.9
Nitrogen, %[a]	0.58	0.21	0.48	0.22	0.87	0.26	0.44	0.36	0.46	0.35
Protein, %[a]	3.63	1.31	3.00	1.38	5.44	1.63	2.75	2.25	2.88	2.16
Methoxyl, %[b]	3.0	3.7	3.2	3.4	2.0	3.1	0.4	0.7	0.3	1.2
Intrinsic[a] viscosity ml g^{-1}	np	700	np	590	np	500	np	60	np	69

Footnotes : a : corrected for moisture content
b : corrected for moisture and protein content
np : not possible; no effective solvent available

alkaline solvents; urea; guanidine hydrochloride; dimethyl sulfoxide and N-methyl morpholine N-oxide (monohydrate), but they were unsuccessful.

On acid hydrolysis, both components yielded D -galacturonic acid, D -galactose, L -arabinose, D -xylose, L -fucose, L -rhamnose and traces (<1%) of D -glucuronic acid and D -glucose. There were no major differences in the sugar contents of the water-insoluble and water-soluble components of Iranian samples 1, 3, 4 and 7.

Amino acid profiles for the water-insoluble and water-soluble components of gum tragacanth samples 4, 5, 6 and 7 from Iran are shown in Figures VII.3 to VII.6. The most prominent amino acids in all the components were hydroxyproline, serine, valine and proline, to which can be added histidine (in both components of sample 4 and the water-insoluble components of samples 5, 6 and 7) and aspartic acid (in the water-soluble components of samples 5, 6 and 7). The water insoluble components were found to have consistently greater proportions of histidine, hydroxyproline and tyrosine than the water-soluble components. Aspartic acid was always present in greater proportions in the water-soluble components than the water-insoluble components.

The amino acid profiles for the Turkish gum tragacanth samples 8, 11 and the sample from Astragalus kurdicus are detailed in Figures VII.7, VII.9 and VII.11.

Hydroxyproline, serine and valine were present in high proportions in all the components of these three samples, together with proline and histidine in both components of sample 8; proline and histidine in the water-insoluble component of sample 9; proline, histidine and aspartic acid in the water-soluble component of sample 9 and histidine and aspartic acid in both components of the sample from Astragalus kurdicus.

In comparison with the water-soluble components, the water-insoluble components had increased contents of hydroxyproline, lysine and phenylalanine in sample 8; increased hydoxyproline, isoleucine, leucine, lysine, phenylalanine, serine, threonine, tyrosine and valine in sample 11 and arginine, isoleucine, leucine, lysine, phenylalanine, threonine and tyrosine in the sample from Astragalus kurdicus. There are therefore some slight differences, but also strong similarities between the amino acid contents of the two components.

It has been established that the carbohydrate and protein contents of the water-insoluble and water-soluble components of the samples studied were very similar. It was reported (20, 45) that fractionation of both components of gum tragacanth gave rise to samples with similar sugar contents. This is supported by the results obtained here.

The fact that one component of gum tragacanth is water-insoluble and the other component is water-soluble is not explained

by an substantial differences in composition and structural differences between then appear to be involved.

Insoluble and soluble hydroxyproline-containing glycoproteins exist as important structural constituents of the plant cell wall. They have unfortunately, not been investigated extensively to determine their structural similarities or differences (50).

VII.5 CONCLUSIONS

1. Seven commercial Iranian gum tragacanth samples were found to vary in their ash, protein, methoxyl and sugar contents, viscosity and in the ratio of their water-insoluble and water-soluble components. Their amino acid contents did not differ extensively.

2. Five commercial Turkish gum tragacanth samples showed less variation than the Iranian samples and were of generally lower viscosity. Their amino acid contents were similar to those of the Iranian samples.

3. Gum tragacanth samples from Astragalus kurdicus and Astragalus gummifer and a sample of 'Traganton' from Turkey were shown to be similar in composition and properties.

4. A gum tragacanth sample from Astragalus microcephalus from Turkey was shown to differ analytically from 'Traganton' and the Turkish samples from Astragalus kurdicus and

Astragalus gummifer.

5. The Test Article used in a dietary study of gum tragacanth in Man (47) was shown to have been well chosen in order to represent a sample of fair average quality.

6. The water-insoluble and water-soluble components of the gum tragacanth samples studied were found to have similar sugar and amino acid compositions, although the water-insoluble components had lower ash and methoxyl contents and higher protein contents, than the water-soluble components. An explanation has been found for the variation in the ratio of water-insoluble and water-soluble components reported by earlier workers.

FIGURE VII.1 : Key to amino acids

Ala : Alanine

Arg : Arginine

Asp : Aspartic acid

Cys : Cystine

Glu : Glutamic acid

Gly : Glycine

His : Histidine

Hyp : Hydroxyproline

Ile : Isoleucine

Leu : Leucine

Lys : Lysine

Met : Methionine

Phe : Phenylalanine

Pro : Proline

Ser : Serine

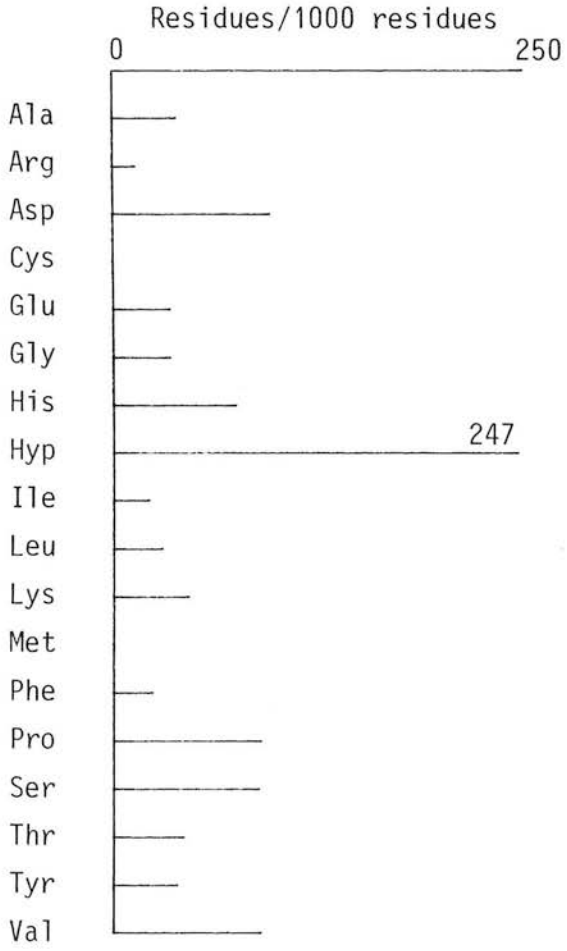
Thr : Threonine

Tyr : Tyrosine

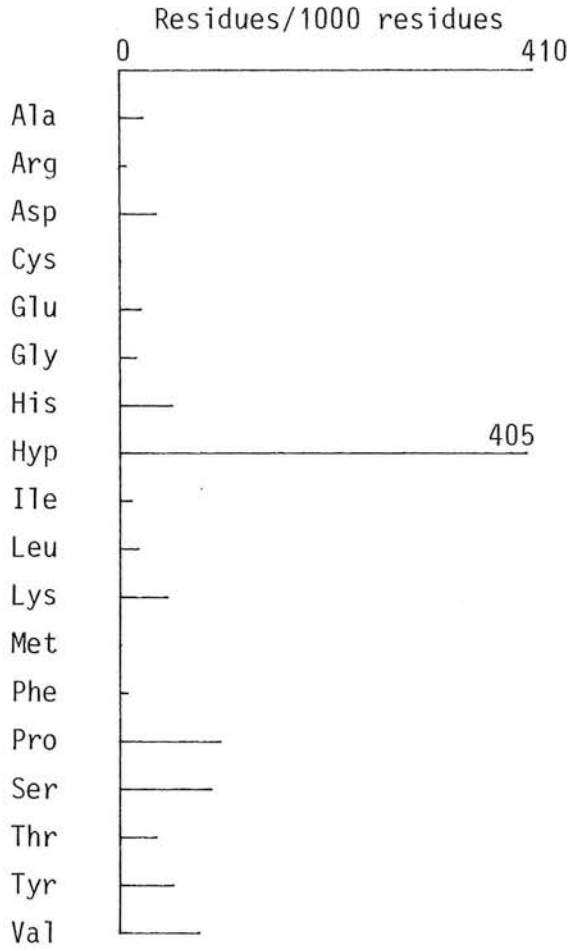
Val : Valine

FIGURE VII.2 : Amino acid profiles

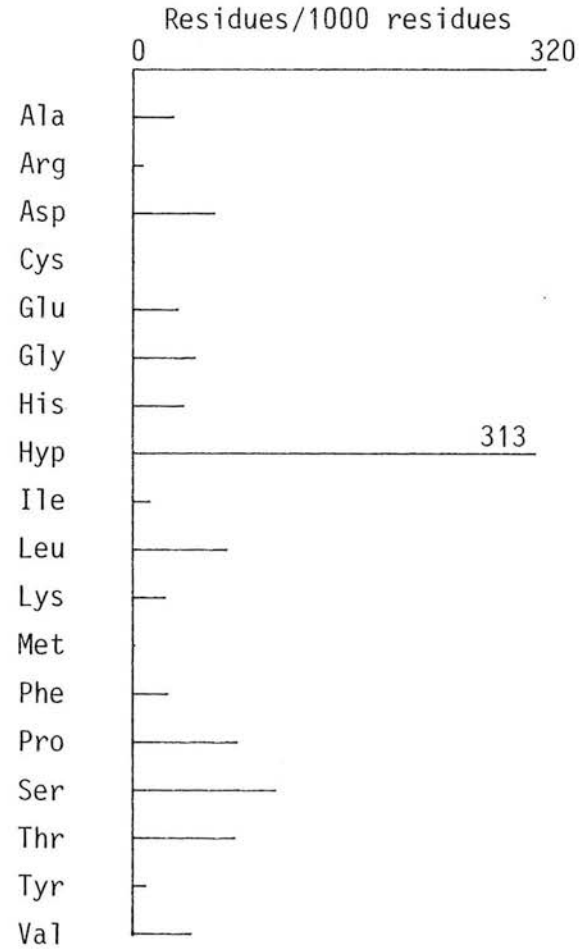
(1) Gum tragacanth 1



(2) Gum tragacanth 3



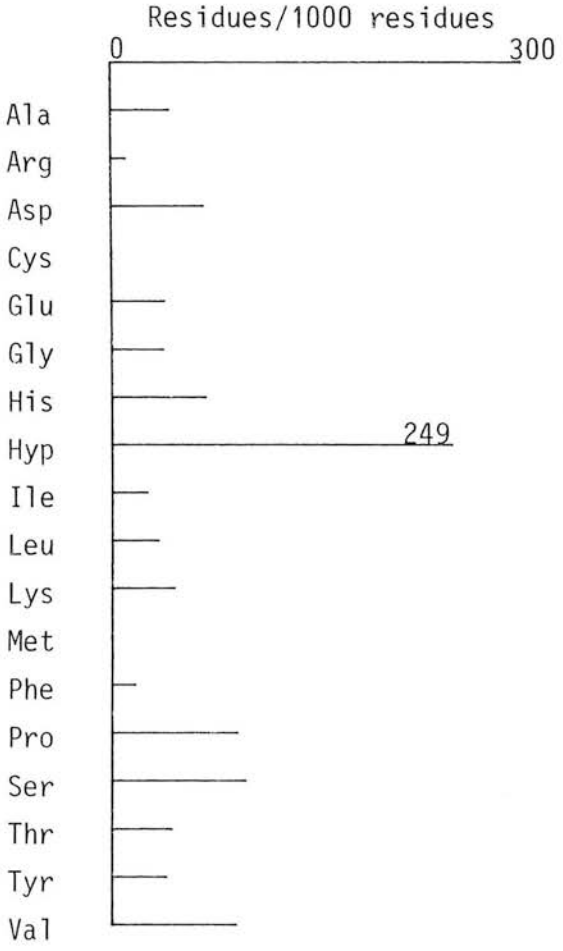
(3) Gum arabic



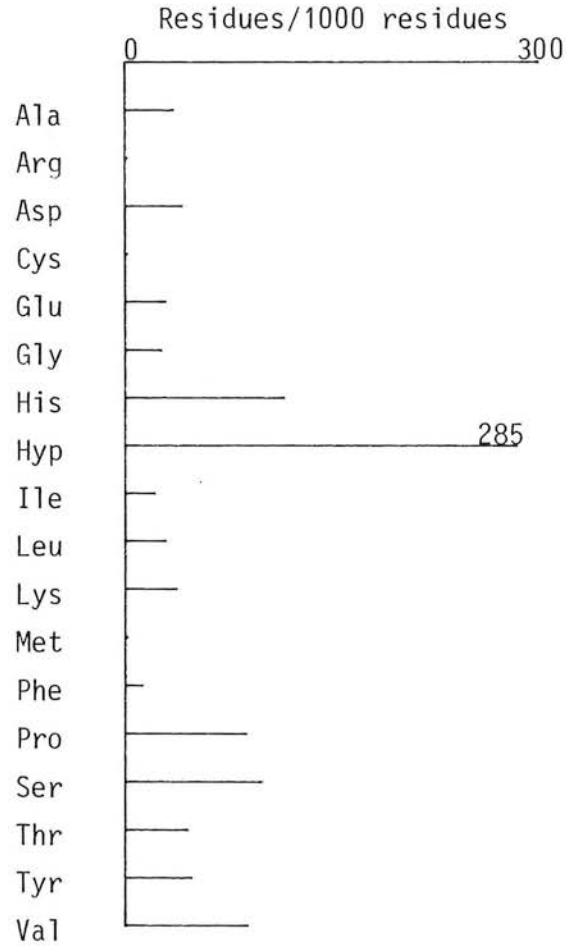
Major amino acids : (1) Hyp Asp Pro Val Ser (2) Hyp Pro Ser Val His (3) Hyp Ser Pro Leu Asp
Footnote : key to amino acids in Figure VII.1.

FIGURE VII.3 : Amino acid profiles

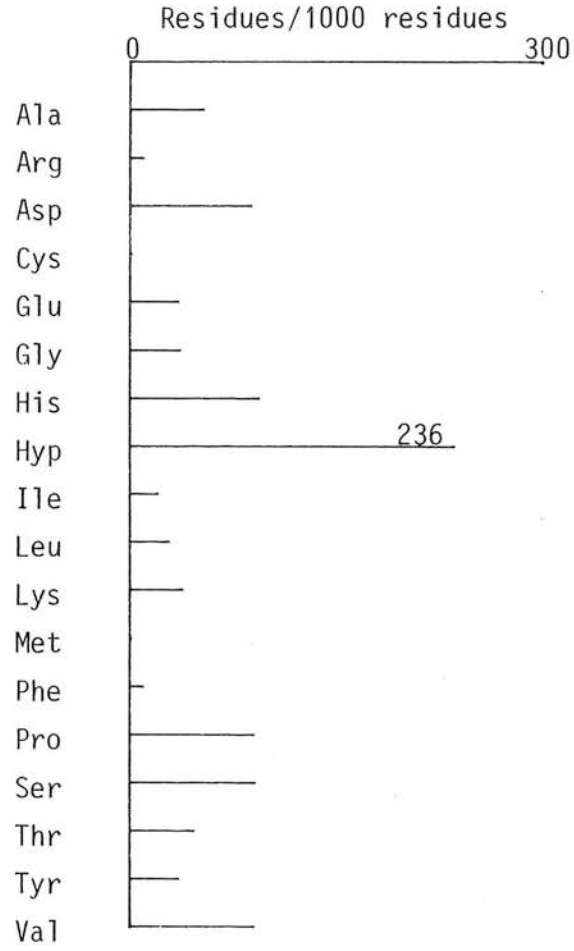
(1) Gum tragacanth 4



(2) Water-insoluble component



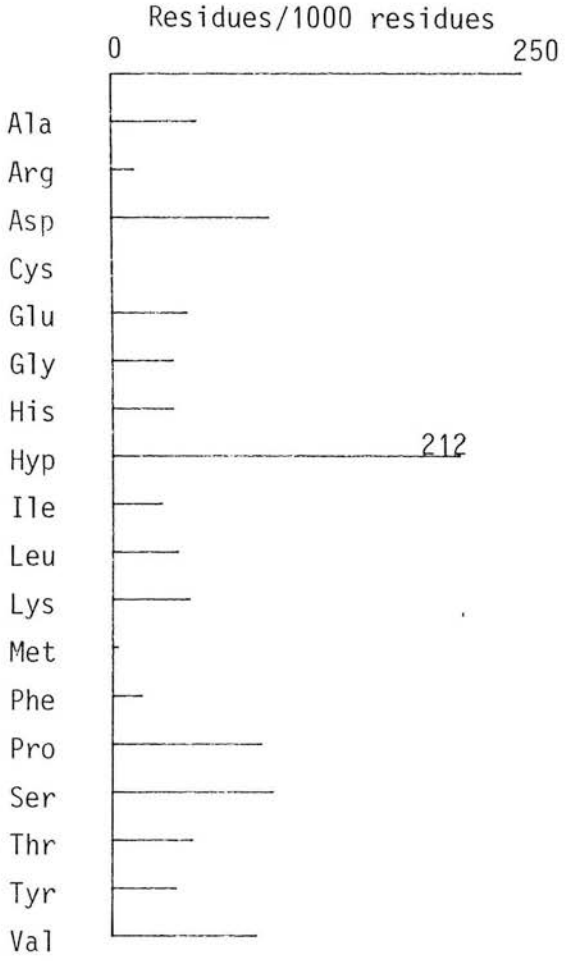
(3) Water-soluble component



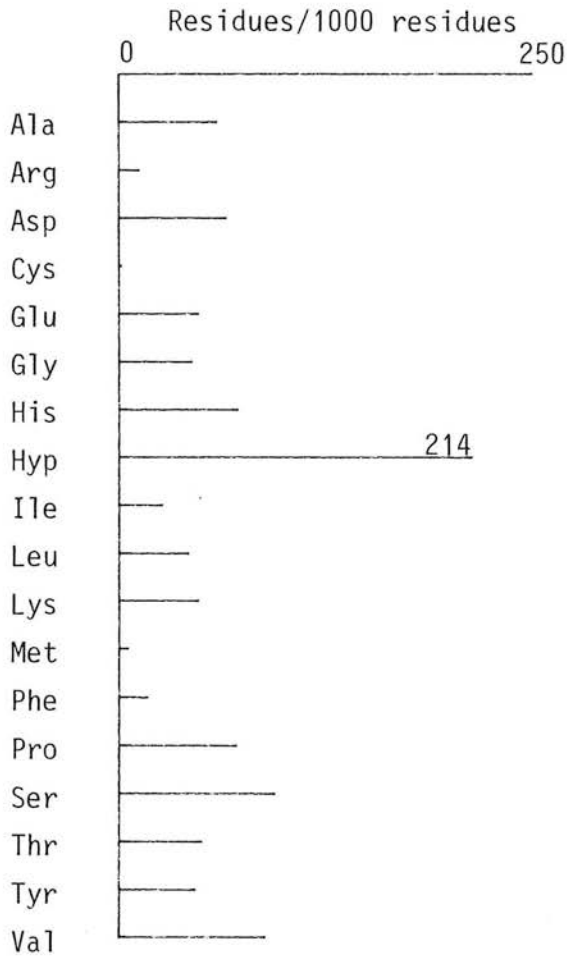
Major amino acids : (1) Hyp Ser Pro Val His (2) Hyp His Ser Val Pro (3) Hyp His Ser Pro Val
Footnote : key to amino acids in Figure VII.1.

FIGURE VII.4 : Amino acid profiles

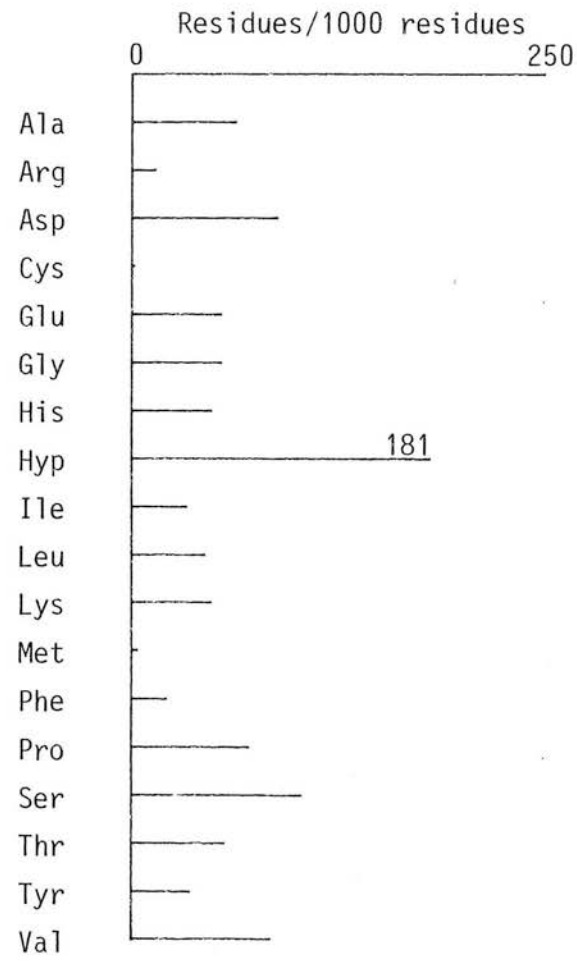
(1) Gum tragacanth 5



(2) Water-insoluble component



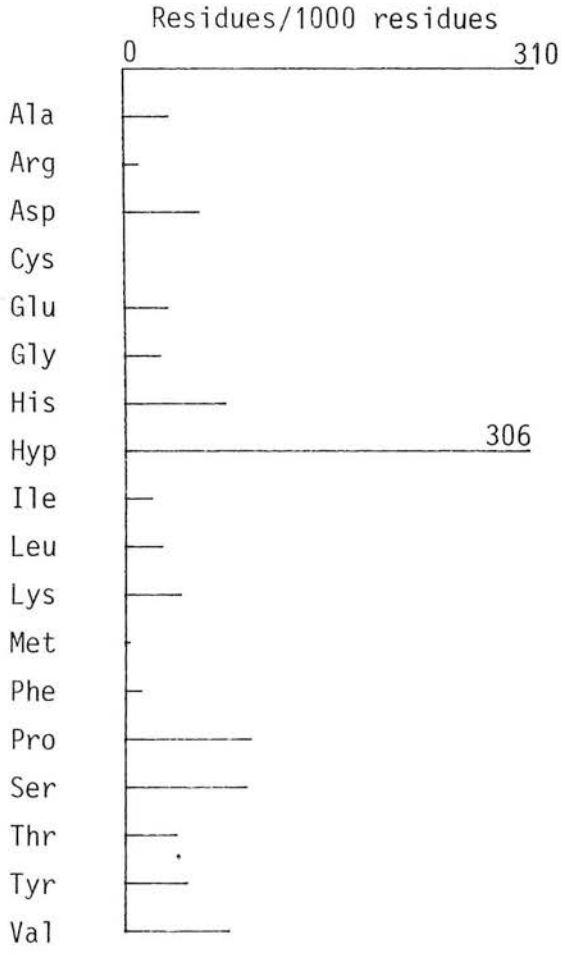
(3) Water-soluble component



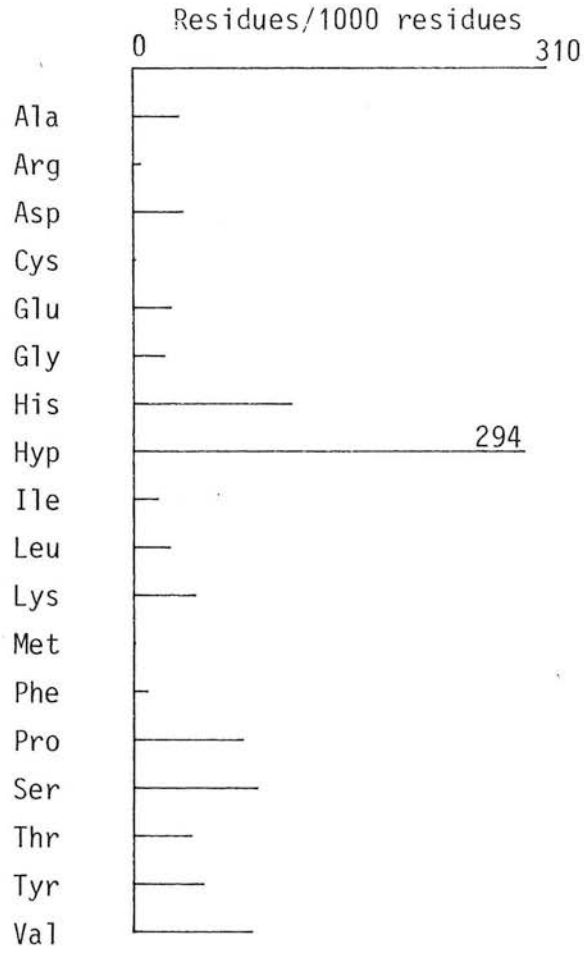
Major amino acids : (1) Hyp Ser Asp Pro Val (2) Hyp Ser Val His Pro (3) Hyp Ser Asp Val Pro
Footnote : key to amino acids in Figure VII.1.

FIGURE VII.5 : Amino acid profiles

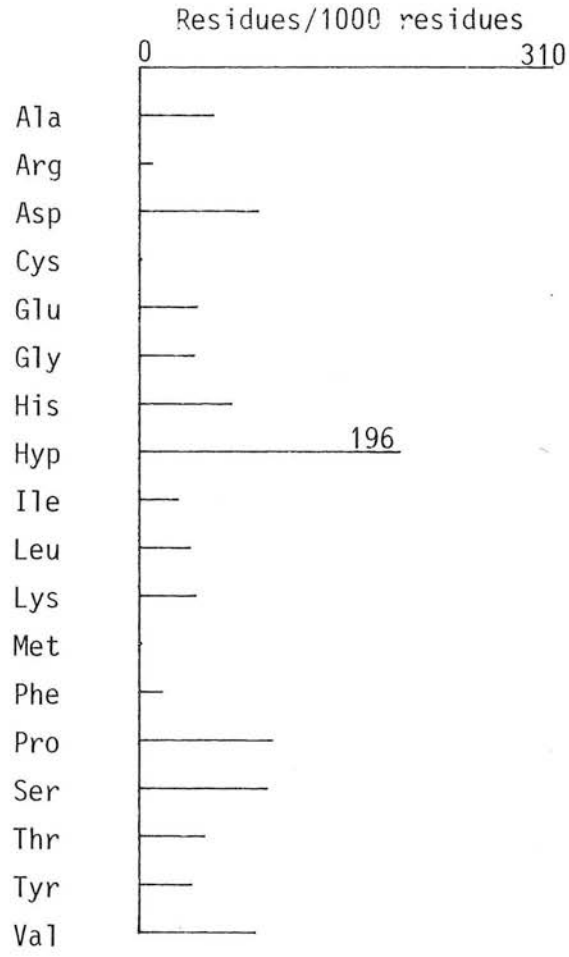
(1) Gum tragacanth 6



(2) Water-insoluble component



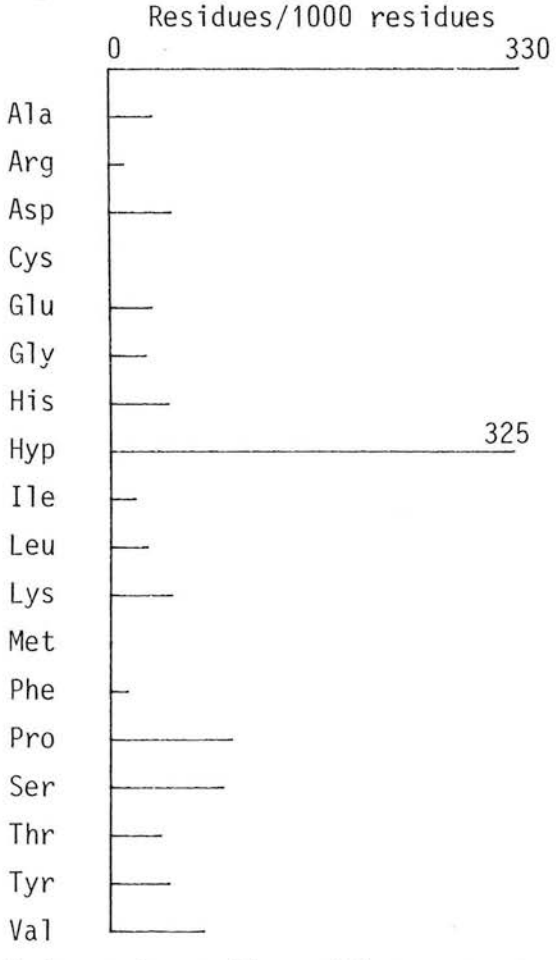
(3) Water-soluble component



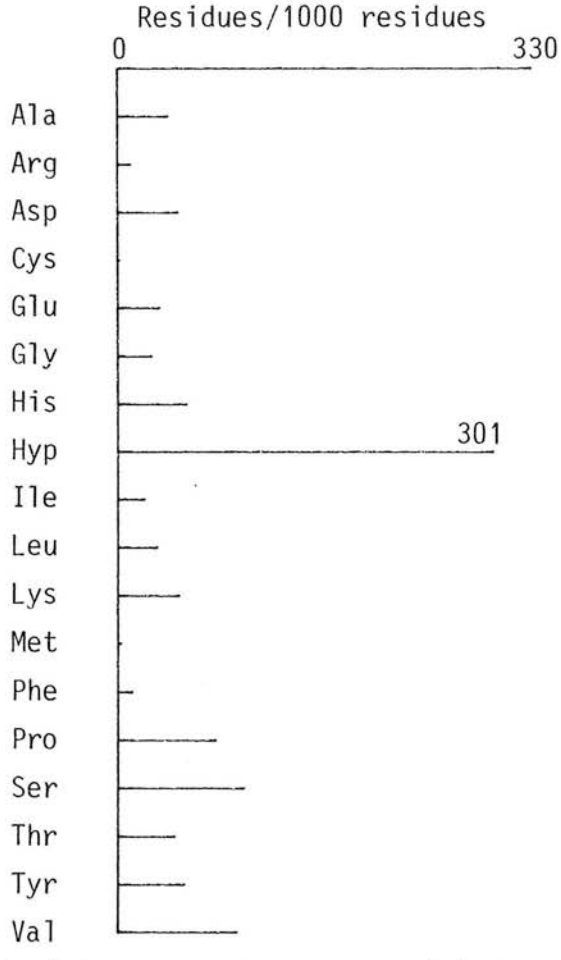
Major amino acids : (1) Hyp Pro Ser Val His (2) Hyp His Ser Val Pro (3) Hyp Pro Ser Asp Val
Footnote : key to amino acids in Figure VII.1.

FIGURE VII.6 : Amino acid profiles

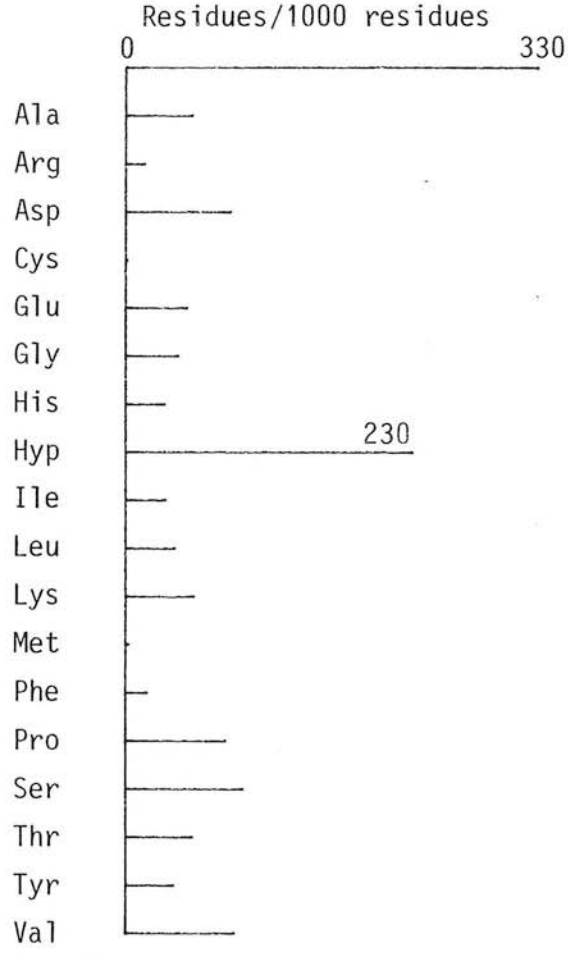
(1) Gum tragacanth 7



(2) Water-insoluble component

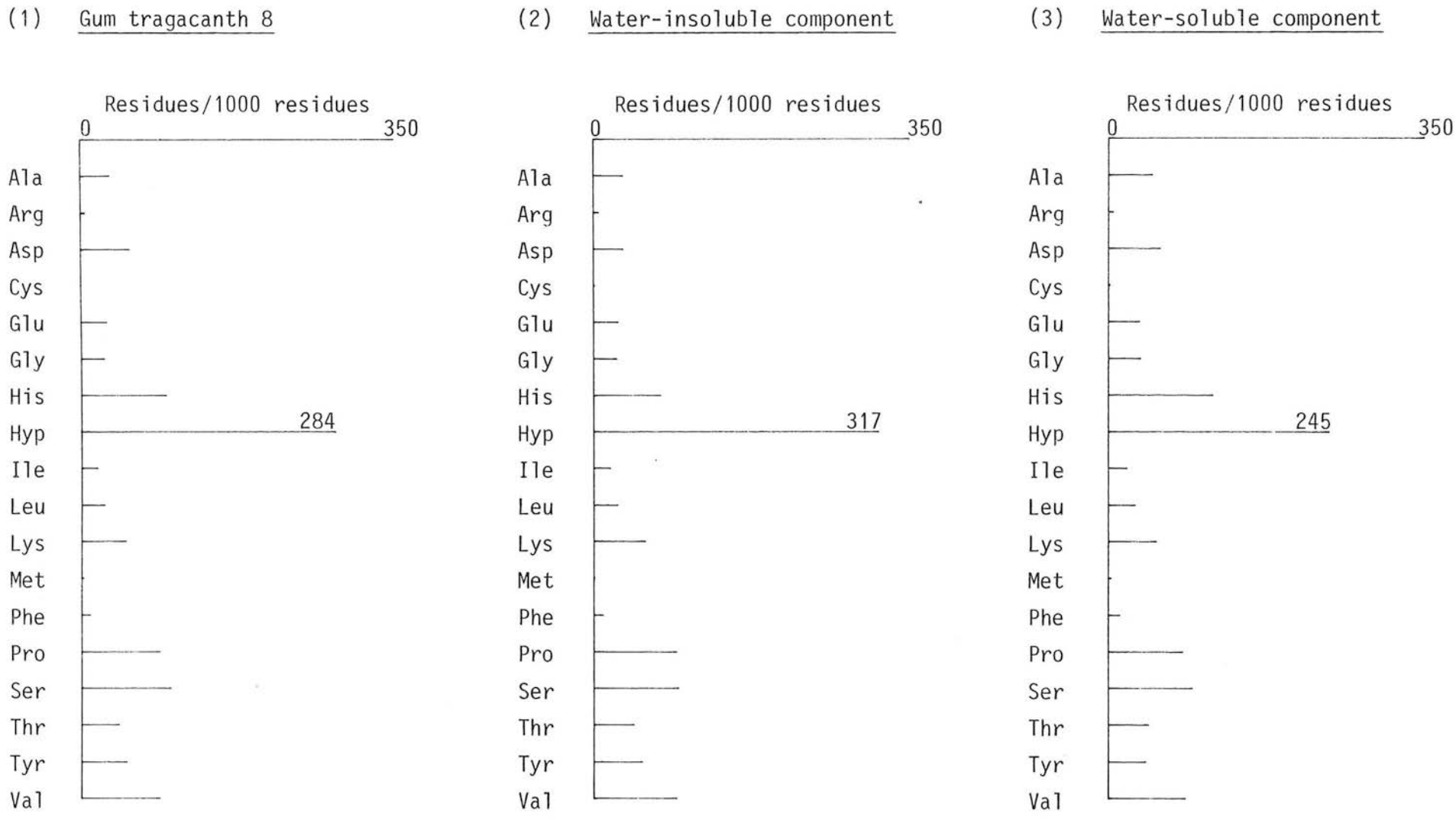


(3) Water-soluble component



Major amino acids : (1) Hyp Pro Ser Asp Val (2) Hyp Pro Ser Val Asp (3) Hyp Ser Val Pro His
Footnote : key to amino acids in Figure VII.1.

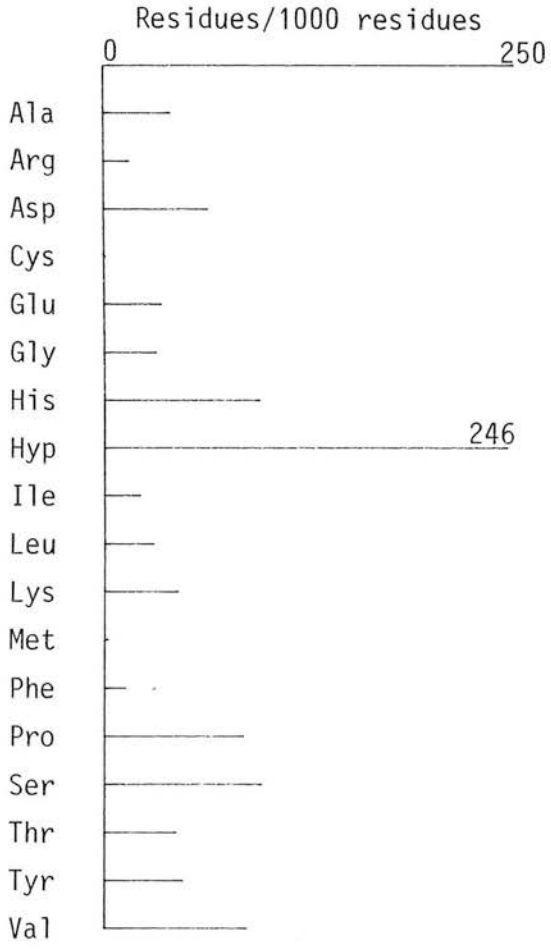
FIGURE VII.7 : Amino acid profiles



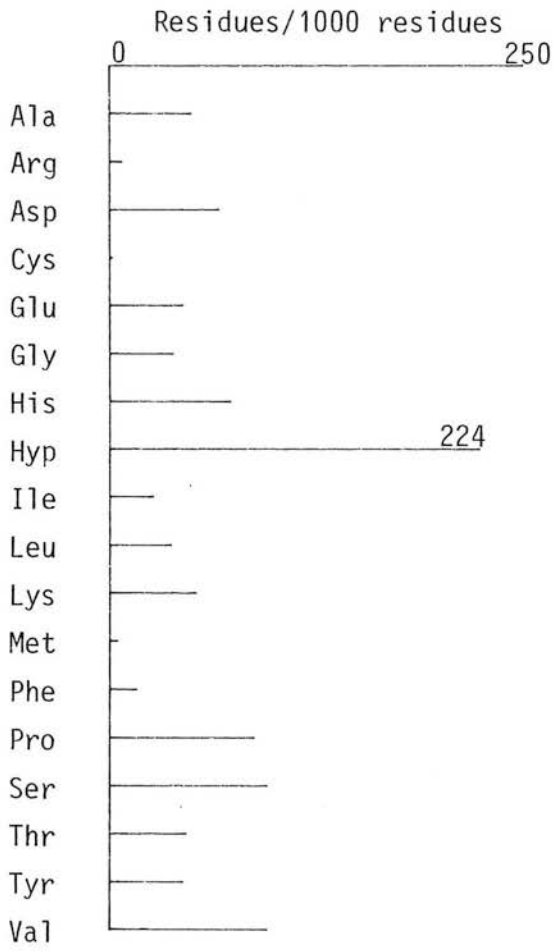
Major amino acids (1) Hyp Ser His Pro Val (2) Hyp Ser Pro Val His (3) Hyp His Ser Val Pro
Footnote : key to amino acids in Figure VII.1.

FIGURE VII.8 : Amino acid profiles

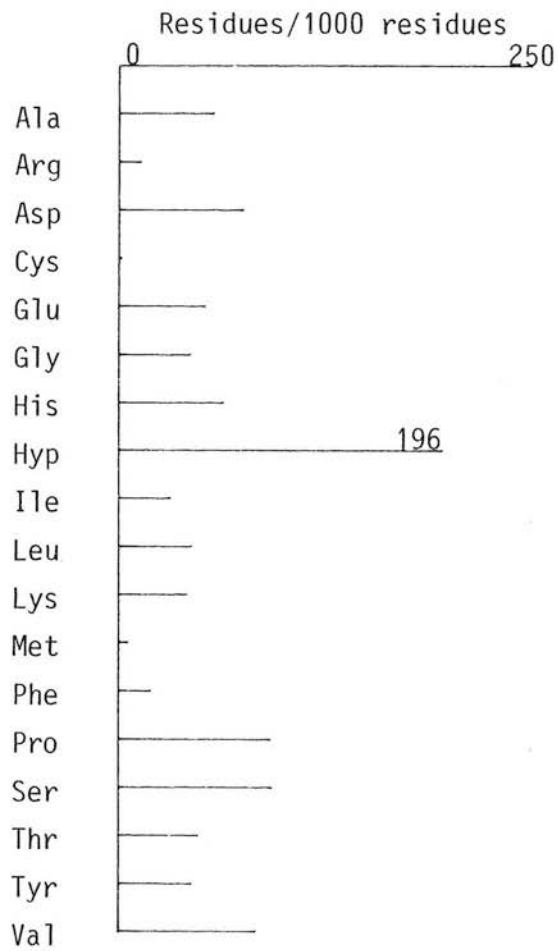
(1) Gum tragacanth 9



(2) Gum tragacanth 10



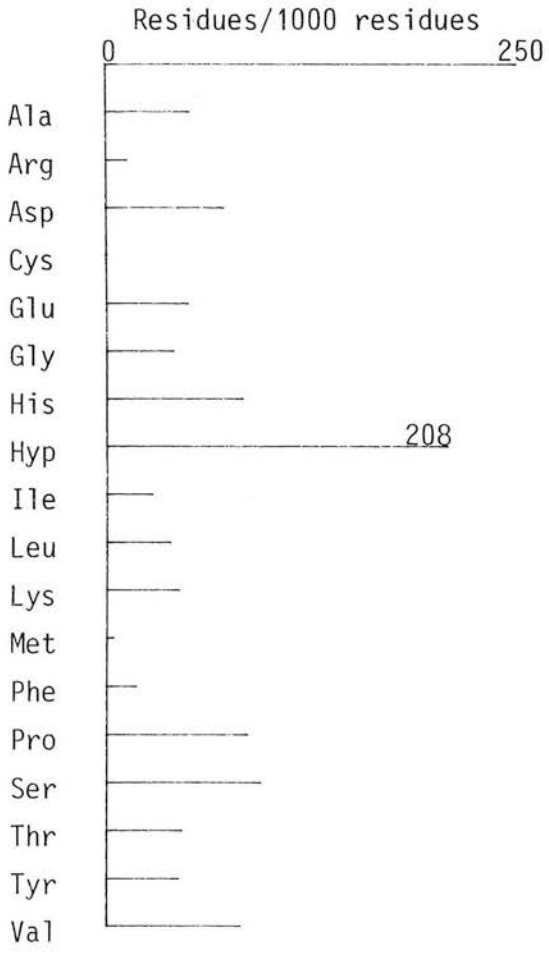
(3) Gum tragacanth 12



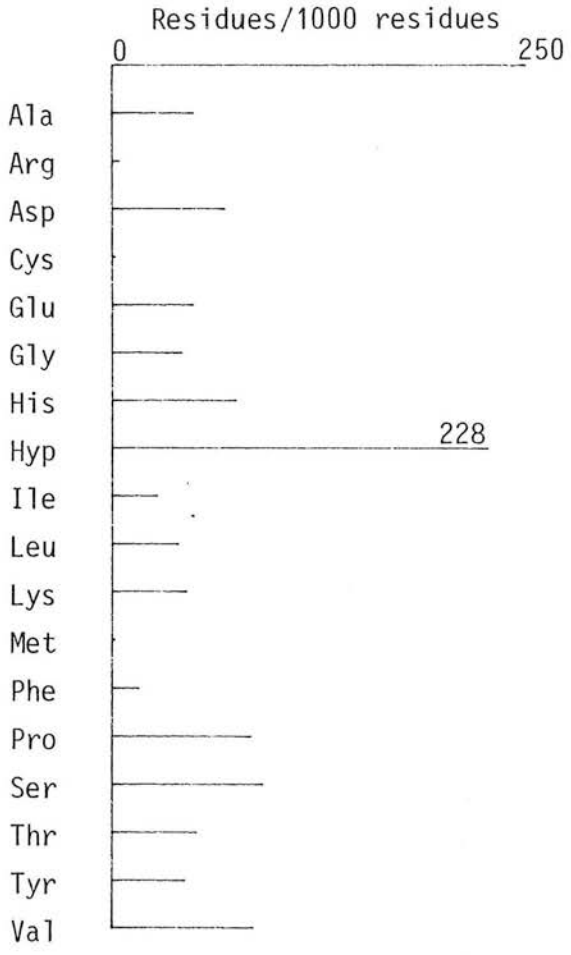
Major amino acids (1) Hyp Ser His Val Pro (2) Hyp Ser Val Pro His (3) Hyp Ser Pro Val Asp
Footnote : key to amino acids in Figure VII.1.

FIGURE VII.9 : Amino acid profiles

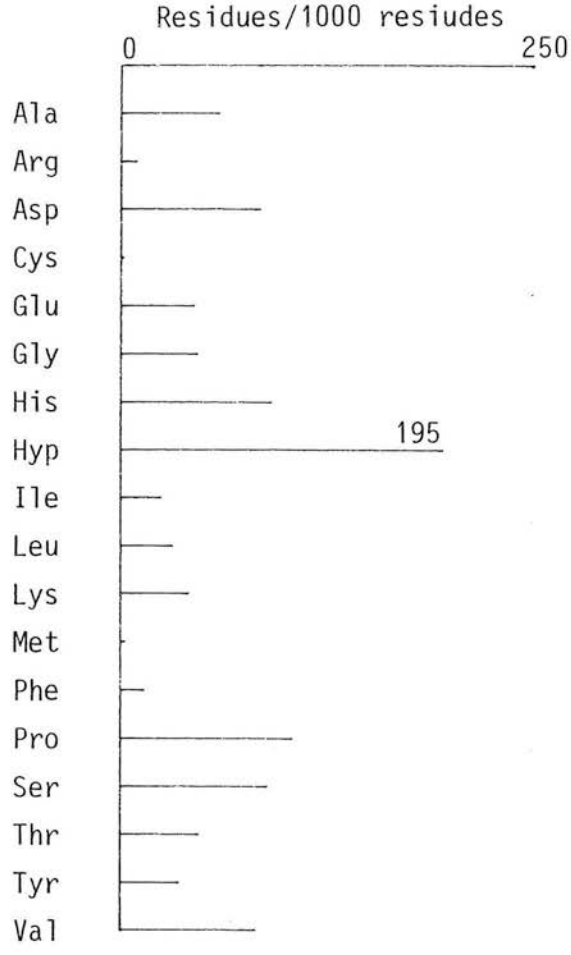
(1) Gum tragacanth 11



(2) Water-insoluble component



(3) Water-soluble component

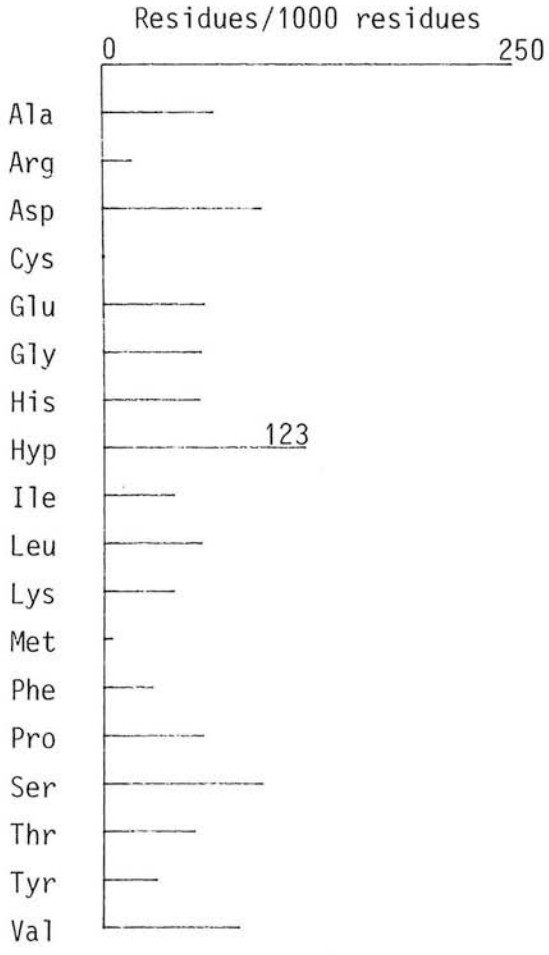


Major amino acids (1) Hyp Ser Pro His Val (2) Hyp Ser Val Pro His (3) Hyp Pro His Ser Asp

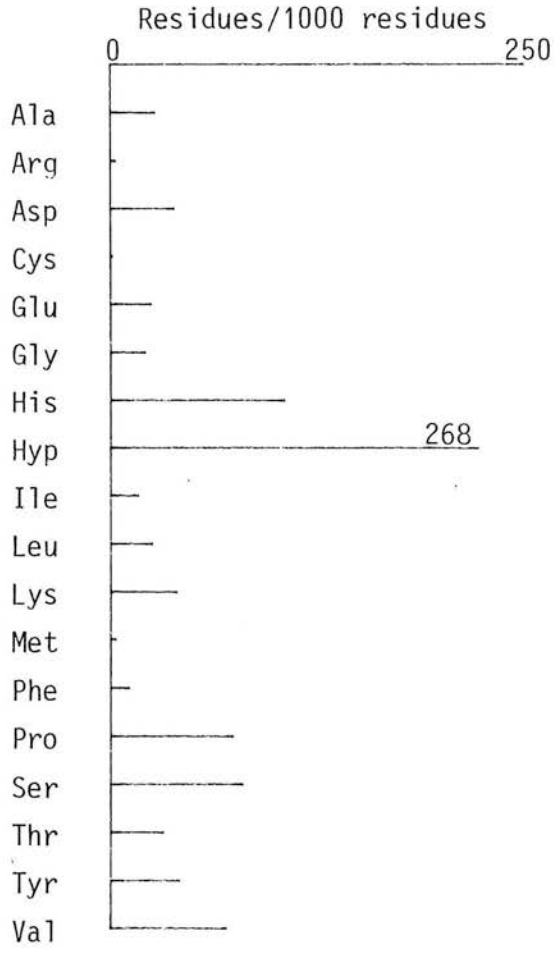
Footnote : key to amino acids in Figure VII.1.

FIGURE VII.10 : Amino acid profiles

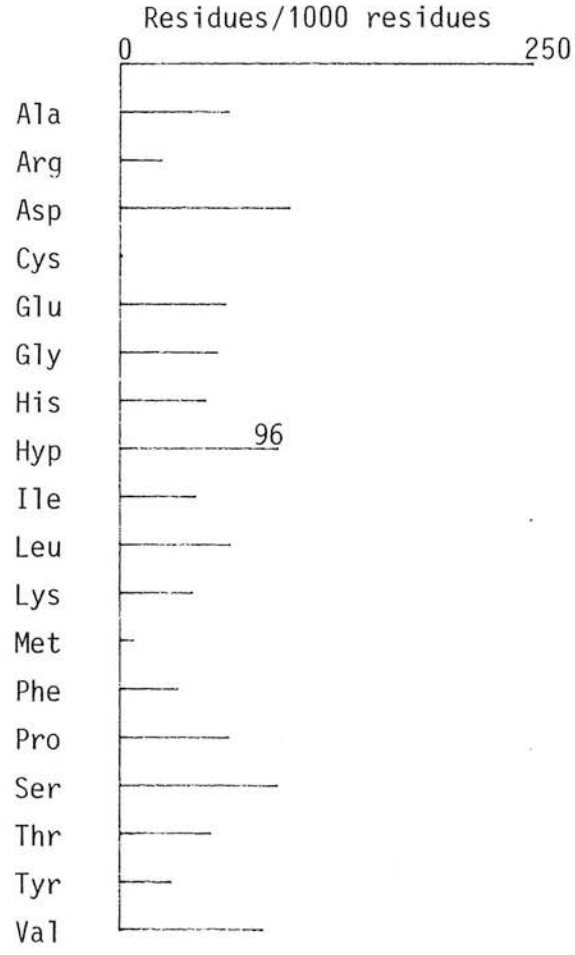
(1) 'Traganton'



(2) Astragalus microcephalus



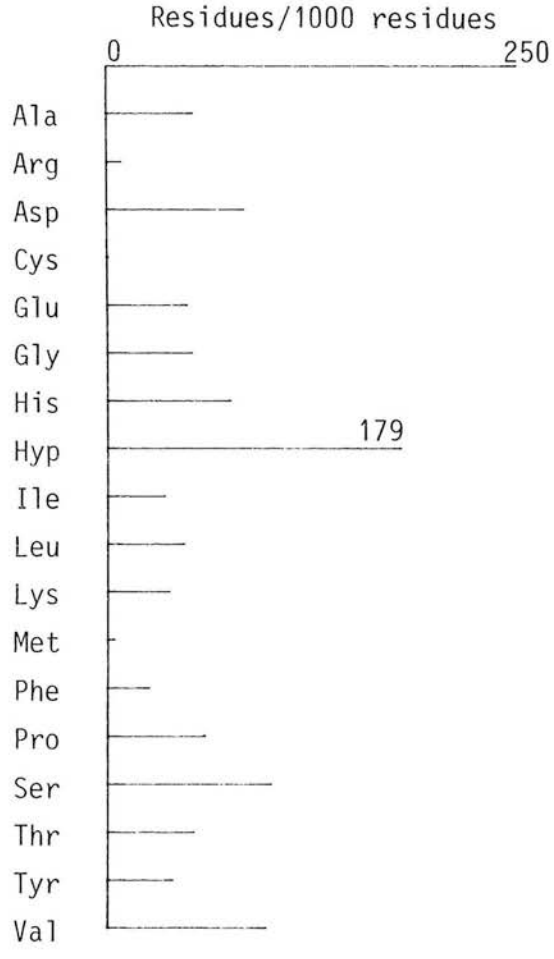
(3) Astragalus gummifer



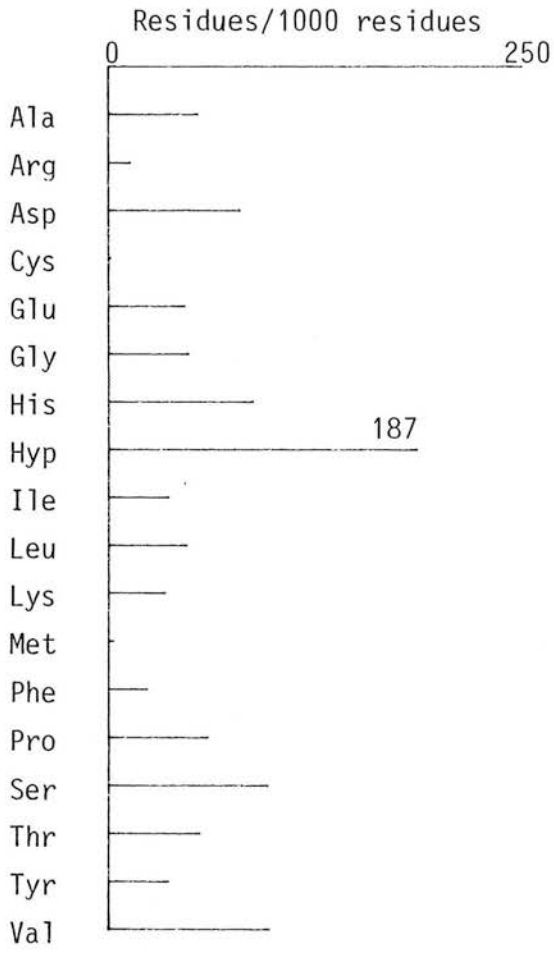
Major amino acids : (1) Hyp Asp Ser Val Ala (2) Hyp His Ser Pro Val (3) Asp Hyp Ser Val Leu
Footnote : key to amino acids in Figure VII.1.

FIGURE VII.11 : Amino acid profiles

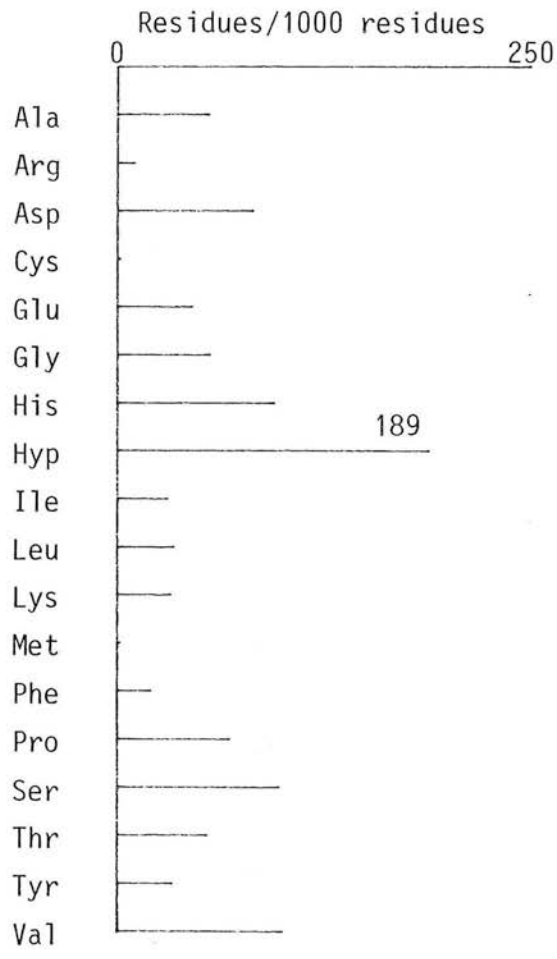
(1) Astragalus kurdicus



(2) Water-insoluble component



(3) Water-soluble component



Major amino acids : (1) Hyp Ser Val Asp His (2) Hyp Val Ser His Asp (3) Hyp Val Ser His Asp
Footnote : key to amino acids in Figure VII.1.

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The International Tree Crops Journal, 2 (1983) 291-295

STUDIES OF URONIC ACID MATERIALS, PART 59: THE GUM EXUDATE FROM A CULTIVAR OF *LEUCAENA LEUCOCEPHALA* (LAM.) DE WIT

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SUMMARY

The gum exuded by a cultivar of *Leucaena leucocephala* growing in South India has been analysed. Its analytical parameters indicate that it is similar to gum arabic. The physico-chemical properties of aqueous solutions (good solubility, viscosity and colour) suggest that Leucaena gum would have useful commercial applications; its stability in acidic solutions is better than that of gum arabic.

INTRODUCTION

In recent years there has been widespread interest in various aspects of the cultivation of *Leucaena leucocephala* (Lam.) de Wit because of its many attractive ecological properties. It is a valuable source of proteinaceous fodder for animals and edible beans for humans; grows very rapidly yielding good quality timber, wood-pulp or firewood; stabilises soils; and fixes nitrogen symbiotically (Blom, 1981). Of all tropical legumes, leucaena is considered to offer the widest assortment of uses (U.S. National Academy of Sciences, 1977). These have been described fully in other excellent publications (U.S. National Academy of Sciences, 1979 and 1980).

Leucaena leucocephala shows great genetic diversity. At least 100 different varieties are known, based on three main types ascribed to Hawaii, Salvador and Peru. This diversity offers the plant breeder opportunities for eliminating some undesirable attributes (e.g. high mimosine content, toxic to ruminants, in the leaves of some varieties) and for exploiting others (e.g. possible gum production).

Correspondence on gum chemistry with Mr R.M. Eggenberger of Matrimandir Peace Gardens, part of an impressive land reclamation project being undertaken by an international community of people at Auroville, near Pondicherry in South India led him to send for examination a sample of the gum exudate given by a cultivar of *Leucaena leucocephala,* grown from seed provided by Dr J.L. Brewbaker, University of Hawaii. The tree involved, about 2.5 years old, produced gum copiously. It did not appear to be diseased in any way; none of the other cultivars of leucaena at Auroville yielded gum.

ANALYTICAL METHODS

Standard analytical methods in gum chemistry (Anderson et al., 1972) were used. Some of the analyses (determination of moisture and ash contents; viscosity measurements; establishing conditions for complete and partial hydrolyses; the identification of neutral and acidic sugars after hydrolysis) were done at George Watson's College, the remainder at Edinburgh University.

RESULTS

The analytical data obtained are given in Table 1.

Partial hydrolyses (0.01M and 0.05M sulphuric acid at 100°C for 16 h) gave considerable amounts of arabinose; for complete hydrolysis, 1.2M sulphuric acid at 100°C for 16 h was required. The gum is, therefore, rather more stable in acidic solution than gum arabic. The crude gum was pale yellow in colour, free from bark and sand; it dissolved readily overnight in cold water to give almost colourless solutions free from insoluble gel.

DISCUSSION

The genus *Leucaena* belongs to the Leguminosae, sub-family Mimosoideae, and therefore has a close relationship with other well-known gum-bearing genera e.g. *Acacia, Prosopis, Albizia.* It was therefore of interest to find out if the exudate from this cultivar of *Leucaena leucocephala* bore similarities in composition and properties to any of the gum exudates from other genera in Mimosoideae studied previously. It has been recorded (U.S. National Academy of Sciences,

1977, p. 74, p. 87) that leucaena seeds contain a water-soluble galactomannan; this will, presumably, have properties of the type given by aqueous extracts of the seeds from other industrially important legumes (guar gum, locust bean gum).

TABLE 1
Analytical Data for the Gum Exudate from a Cultivar of *Leucaena leucocephala* (corresponding data (Anderson et al, 1966, 1968) for gum arabic given in parentheses).

Moisture, %	17.7	(13.1)
Ash, %[a]	4.4	(3.8)
Nitrogen, %[b]	0.37	(0.37)
Hence protein, % (N x 6.25)[b]	2.3	(2.3)
Equivalent weight[b]	767	(1085)
Hence uronic anhydride, %[c]	23	(16)
Methoxyl, %[b]	0.92	(0.25)
Specific rotation $[\alpha]_D$, degrees[b,d]	−28	(−30)
Intrinsic viscosity $[\eta]$, ml/g[b,e]	24.1	(13.4)
Molecular weight, $\bar{M}_w$ [b,e]	1.7×10^6	(0.58×10^6)
% Sugar composition after hydrolysis:−[f]		
Glucuronic acid	17.5	(16.0)
4-0-Methylglucuronic acid[g]	5.5	(1.5)
Galactose	36	(40)
Arabinose	22	(28)
Rhamnose	19	(14)

Footnotes

[a] corrected for moisture content; [b] corrected for moisture and ash contents; [c] if all acidity arises from uronic acid groups; [d] C, 1.0% in water; [e] in 1.0M sodium chloride; [f] corrected for moisture, ash and protein contents; [g] if all methoxyl content present in this acid.

The behaviour of this gum in aqueous and acid solution is similar to that of gum arabic. The neutral and acidic constituent sugars of *Leucaena leucocephala* gum are identical to those found in *Acacia* spp. gum exudates but differ slightly from those found in *Prosopis* spp. gums (Anderson and Farquhar, 1982) and *Albizia* spp. gums (Anderson and Dea, 1969). The analytical parameters for *ca.* 100 different exudates of *Acacia spp.* are now known (Anderson, 1978); although these show very wide differences analytically in the proportions of galactose, arabinose, rhamnose and glucuronic acid always present, they can be systematised for general purposes into several groups which follow Bentham's (1875) sub-divisions of the genus remarkably closely. The gum from *L. Leucocephala* has certain similarities (negative specific rotation, low nitrogen content, with a ratio of rhamnose to glucuronic acid close to unity) to the gums from *Acacia* spp. placed in Bentham's (1875) Group V (Vulgares). The commercial source of gum arabic, *A. senegal* Willd., is a member of that group. When compared directly (Table 1) with typical parameters for *A. senegal* gum, this specimen of leucaena gum is more viscous and more acidic, with a higher molecular weight and higher methoxyl and rhamnose contents. It has, therefore, several features that would make it of commercial interest. If a gum-yielding cultivar of *Leucaena leucocephala* could be developed and used in some of the planting projects desirable ecologically, particularly in arid zones, the scale necessary for such operations could lead to production of considerable quantities of *Leucaena leucocephala* gum exudate.

ACKNOWLEDGEMENT

We thank Mr R.M. Eggenberger, Auroville, India for providing the sample of gum, and the Science Research Council for a maintenance award (to M.M.E.B.)

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RÉSUMÉ

La gomme exsudée par un cultivar de *Leucaena leucocephala* croissant dans le sud des Indes a été analysée. Les resultats des analyses indiquent qu'elle est semblable à la gomme arabique. Les caractéristiques physico-chimiques des solutions aqueuses (bonne solubilité, viscosité et coleur) suggèrent que la gomme de leucaena aurait des applications commerciales utiles; sa stabilité dans des solutions acides est meilleur que celle de la gomme arabique.

RESUMEN

La goma exhudada por una variedad de *Leucaena leucocephala* que crece en el sur de India fue analizada. Sus paràmetros analíticos indican que es similar a la goma arábica. Las propiedades físico-químicas de soluciones acuosas (buena solubilidad, viscosidad y color) sugieren que la goma de leucaena podría tener aplicaciónes comerciales de utilidad; su estabilidad en soluciones ácidas es mejor que la de la goma arábica.

The International Tree Crops Journal, 2 (1983) 245-254

THE CHEMICAL CHARACTERIZATION OF THE TEST ARTICLE USED IN TOXICOLOGICAL STUDIES OF GUM ARABIC (*ACACIA SENEGAL* (L.) WILLD).

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SUMMARY

The sample of commercial gum arabic used as the "Test Article" in recent toxicological studies has been characterized chemically. Comparative analytical data, presented for seven other commercial gum arabic samples, and for five specimens of gum collected from authenticated *Acacia senegal* trees in several gum producing countries, indicate that the Test Article is a fair, average representative sample of gum arabic originating from *Acacia senegal* and conforming in all respects to established international standards of identity and purity.

INTRODUCTION

Gum arabic is a valuable tree crop in arid zone developing countries; it was important for its toxicological status to be established so that it can remain an important commodity in international trade. The collection of gum arabic provides the major source of income (some U.S. $ 150) for many Sahelian nomads and remote farmers. World demand reached ca. 70,000 tons in the late sixties, but the Sahelian droughts of the early seventies led to supply shortages, escalating prices, and market uncertainty. However, in the past 5 years, adequate supplies and stabilisation of Sudanese gum export prices have led to a gradual restoration of market confidence and recovery of annual consumption to ca. 40,000 tons (value ca. U.S. $ 60 million). After cotton and oil seeds, exports of gum arabic provide the third largest source of overseas earnings for the Sudan, which produces about 85% of the world's supply. With the aid of internationally sponsored re-afforestation programmes, 1 million *Acacia senegal* trees are now planted each year in selected areas of the Sudan to stabilise soil erosion, halt desert encroachment, and enrich soil

through nitrogen fixation. *Acacia* trees provide forage for grazing animals, employment, a source of income for farmers if the farm gate price for gum remains sufficiently attractive, and an eventual source of fuel-wood when the trees become too old to yield gum adequately. Gum arabic production is therefore important ecologically and economically; the other Sahelian countries and Ethiopia are also striving to increase gum arabic production.

Within the EEC, gum arabic is a permitted foodstuffs additive (labelling code E414) when it conforms to the definition 'a dried gummy exudate from stems and branches of *Acacia senegal* (L.) Willd. or of related species of *Acacia*' (EEC, 1978). The Joint WHO/FAO Expert Committee on Food Additives (JECFA) has, however, world-wide legal powers; its latest specifications for identity and purity (FAO, 1982) define gum arabic as 'a dried exudation from the stems and branches of *Acacia senegal* (L.) Willdenow or the related species of *Acacia* (Fam. Leguminosae). It consists mainly of high molecular weight polysaccharides and their calcium, magnesium and potassium salts, which on hydrolysis yield arabinose, galactose, rhamnose and glucuronic acid. The article of commerce may be further specified as to viscosity'.

Data made available to regulatory authorities in 1981, from toxicological studies in laboratory animals and in humans, allowed JECFA in April 1982 to establish the safety of gum arabic as a foodstuffs additive, classifying it within the category for which the Acceptable Daily Intake (ADI) is deemed to be 'not specified'. This paper presents analytical data which characterize completely the sample of commercial gum arabic (the 'Test Article') used in the following toxicological studies submitted to JECFA and other international regulatory bodies on behalf of commercial sponsors: a metabolic study (Ross et al., 1982) and two separate sub-acute (90-day) toxicity studies (Anderson et al., 1982) in laboratory rats; a dietary study in humans (Ross et al., 1982) and a study by electron microscopy to establish the absence of mitochondrial and other changes in rat heart and liver following ingestion of gum arabic (Anderson et al., 1983).

Previous analytical studies of the exudates from ca. 100 *Acacia* spp. have established that each species yields a unique gum of characteristic chemical composition (Anderson, 1978). Although seasonal and geographical variations are liable to occur in all complex natural products, it has been shown that the seasonal and geographical

variations for *A.senegal* gum are relatively insignificant and very much smaller than the differences in chemical composition between *A.senegal* gum and its closest botanical relatives, of which there are only a few of any practical significance. There are gross differences in composition between *A.senegal* gum and the gums from species (e.g. *A.seyal*) in other sub-divisions of the genus (Anderson, 1978).

In order to gain general international clearance for a natural product exported from so many different countries, it was seen to be important to establish that the Test Article chosen is a representative, typical sample of commercial gum arabic. This has been done by establishing the close similarity of the Test Article, in terms of its analytical parameters and physical properties, to that of seven other reputable commercial samples from the main producing countries. Moreover, as it is of legal importance to establish that the Test Article originated essentially from *Acacia senegal* trees, and not from unrelated *Acacia* spp., analytical data are also presented for five gum specimens, collected from *A.senegal* trees by acknowledged botanical experts in various gum producing countries.

EXPERIMENTAL

Origin of gum specimens and samples

Specimen 1 represents many gum specimens, collected by the late Mr. M.P. Vidal-Hall, Gum Research Officer to the Sudanese Government, from individual trees in forest reserves at Qala en Nahal (Kassalla Province), Umm Ruaba and Goz el Ganzara (Kordofan Province) (Anderson et al., 1968).

Specimens 2, 3 and 4 were collected by Dr. J. Vassal (Université Paul Sabatier, Toulouse) from *A.senegal* trees growing respectively at Dahra, Ferlo, Senegal; Nioro en Sahel, Mali; Timbuctou, Mauritania.

Specimen 5 was collected in Oman by Dr. R.M. Lawton, Forestry Adviser to the Sultanate of Oman.

Samples 6–10 were commercial gum arabic samples obtained respectively from Chad, Ethiopia, Nigeria, Senegal and Sudan, either directly or through European importers.

Samples 11 and 12, kindly provided by Messrs. Rowntree-Mackintosh p.l.c. (York, U.K.) were accumulated, each over 3 months, by pooling the unused portions of samples scrutinised weekly in the quality control laboratory prior to release for normal production purposes. These samples thus reflect the composition of the gum arabic, from a range of provenances, used in foodstuffs production by the major European consumer over a 6-month period.

The Test Article was abstracted from a normal production batch of gum arabic by Messrs. Rowntree-Mackintosh p.l.c. After dissolution of the natural gum, the resulting liquor was freed from sand, tree bark etc. by sieving and centrifugation processes; the gum was recovered by freeze-drying so that an analytically homogeneous bulk sample, sufficient for several large-scale toxicological tests, was secured.

Analytical methods

The standard analytical methods for gum exudates have been described (Anderson et al., 1966, 1972).

RESULTS

The analytical data obtained, and the derived mean values are shown in Table 1.

DISCUSSION

The analytical values for specimen 1 are themselves averages derived from the results for many individual Sudanese specimens. The analyses for specimens 2–5 and samples 6–12 were carried out as replicates after grinding the crude gum finely to try to ensure homogeneity, which is difficult to achieve for samples comprising many individual gum nodules. The sampling problem was particularly acute for the large samples 11 and 12, each representative of several thousands of tons of gum from many provenances. After thorough mechanical mixing, over 2 days, 4 sub-samples (sample 11) and 7 sub-samples (sample 12) were obtained by recognised procedures. As these sub-samples nevertheless showed slight but real analytical variations, the data quoted for samples 11 and 12 are mean values derived from the data for the sub-samples.

TABLE 1
Analytical data for *Acacia senegal* gum specimens and commercial gum arabic samples from various locations

	A.senegal gum specimens				commercial gum arabic samples								Average	Gum arabic
	1	2	3	4	5	6	7	8	9	10	11	12	1–12	'Test Article'
Loss on drying, 105°C, %	(7.0)[e]	13.9	12.9	13.8	9.2	13.5	13.1	13.6	16.4	16.4	13.6	13.3	13.6	(6.0)[e]
Total ash, 550°C, %[a]	3.9	3.2	4.1	3.9	5.1	3.6	3.8	3.9	4.3	4.4	4.1	4.9	4.1	3.0
Nitrogen, %[a]	0.29	0.38	0.23	0.24	0.14	0.58	0.28	0.46	0.35	0.32	0.32	0.39	0.33	0.31
Hence protein (Nx6.25), %[a]	1.8	2.4	1.4	1.5	0.9	3.6	1.7	2.9	2.2	2.0	2.0	2.4	2.0	1.9
Methoxyl, %[b]	0.25	0.23	0.29	0.28	<0.1	0.18	0.14	0.26	0.20	0.14	0.37	0.52	0.26	0.26
Sp. rotation, $[\alpha]_D$, degrees[b]	−30	−33	−29	−25	−40	−32	−29	−31	−33	−32	−32	−29	−31	−30
Intrinsic viscosity, ml/g[a]	13.4	18	14	19	14	20	19	17	19	17	17	18	17	17
Molec. weight ($\bar{M}w$) x 10^5 [a]	5.8	5.5	6.0	6.1	4.6	6.4	11	8.3	6.6	5.0	4.8	5.1	6.3	5.8
Neut. equiv. (electrodialysis)[b]	1100	1260	1090	1260	960	1270	970	1000	870	990	860	890	1040	1020
Hence uronic anhydride, %[b, c]	16	14	16	14	18	14	18	18	20	18	20	20	17	17
Sugar composition after hydrolysis, %														
4-0-methylglucuronic acid[d]	1.5	1.5	1.5	1.5	0	1	1	1.5	1	1	2	3	1.4	1.5
Glucuronic acid	14.5	12.5	14.5	12.5	18	13	17	16.5	19	17	18	17	15.8	15.5
Galactose	44	50	46	53	41	46	46	45	43	46	44	46	46	45
Arabinose	27	25	25	23	27	27	26	27	21	22	21	22	24	24
Rhamnose	13	11	13	10	14	13	10	10	16	14	15	12	13	14

Footnotes: [a]corrected for moisture content; [b]corrected for moisture and protein content; [c]if all acidity arises from uronic acids; [d]if all methoxyl groups located in this acid; [e]value for freeze-dried sample.

This preliminary heterogeneity and sampling difficulty led to the decision that, for intended use in several distinct toxicological tests, the eventual Test Article must be analytically homogeneous; it therefore had to be recovered from solution. This also had the merit of satisfying the requirement (FAO, 1982) that 'the crude gum must be cleaned before use in foods' (i.e. freed from contaminating bark and sand).

The most marked fluctuations in the analytical parameters involve the nitrogen contents of specimen 5 and sample 6 and the specific rotations of specimens 4 and 5.

The rhamnose contents of samples 7 and 8, although lower than average, are not abnormal (Anderson and Dea, 1968).

Overall, therefore, for a complex natural product from a wide array of geographical locations, the range of value established for each analytical parameter must be regarded as surprisingly small. Although wider variations may occur in single authentic nodules, *Acacia senegal* gum has a unique set of analytical parameters by which it can be distinguished from the exudates from other *Acacia* spp. and from other genera. Even the gums exuded by the closest botanical relatives of *A.senegal* have markedly different chemical compositions, viz. *A.laeta* (Anderson and Smith, 1967), *A.mellifera* (Anderson and Farquhar, 1979) and *A.polyacantha* syn. *campylacantha* (Anderson and Munro, 1970). In the opinion of international authorities on the taxonomy of *Acacia* spp., only *A.asak,* in addition to the three species named above, can be regarded as a close relative of *A.senegal* (Elamin, 1983; Wickens, 1983). Analytical data for the gum from *A.asak* are not available.

Botanically, there are many recognisable variants of *Acacia senegal* which, as a result of constant re-shuffling of genes, is best regarded as a broad botanical complex (Brenan, 1968; Anderson et al., 1968). This is the factor involved in longstanding Sudanese tree-improvement programmes: some *A.senegal* trees yield good quality gum copiously; some do not. Gum specimen 5 was collected in Oman from trees recognised by Dr. Lawton as belonging to a local variant of *A.senegal* having some slightly atypical morphological features. A study of this variant, in terms of the sub-species of *A.senegal* described to date, is in progress at The Herbarium, Royal Botanic Gardens, Kew. The specific rotation and nitrogen content of this sample fall outside the normal range of values, but all its other analytical values are so typical that there is no difficulty in accepting this gum as being

derived from *A.senegal* or, at worst, from a very closely related sub-species.

In effect, apart from the gum from *A.senegal* (true gum arabic), only two *Acacia* gums are marketed in commercial quantities. *A.seyal* Del., the source of 'gum talha', has long been recognised as giving an inferior gum which has always been sold as a separate Sudanese commodity. The gum from *A.drepanolobium* Harms ex Sjostedt, dark in colour and partially insoluble, contributes to some East African gum parcels. In all published botanical classifications and revisions of the genus *Acacia, A.seyal* and *A.drepanolobium* are assigned to a different sub-division from *A.senegal* and thus cannot be regarded as related species. The analytical parameters of *A.seyal* gum (Anderson et al., 1969) and of *A.drepanolobium* gum (Anderson and Dea, 1967) differ greatly from those of *A.senegal* gum and are readily distinguishable on account of their highly positive optical rotations. Samples of gum arabic with specific rotations more positive than -25° or -26° must always be suspected to contain *A.seyal, A.drepanolobium,* or other unrelated gums in admixture with *A.senegal.* Good commercial samples tend to show mean values.

In addition to the analytical data in Table 1, the Test Article conformed in all respects to all other tests of purity and identity specified by JECFA (FAO, 1982) and to all the other limits (e.g. sulphated ash, freedom from foreign and insoluble matter, freedom from starch, tannins, heavy metals etc., etc.) laid down in the Food Chemical Codex, B.P., E.P., and U.S. Pharmacopoeias etc.

CONCLUSIONS

The data in Table 1 show that the Test Article used in recent toxicological studies of gum arabic has analytical parameters that correspond closely with those for authenticated specimens of *Acacia senegal* gum and with those for reputable commercial samples of gum arabic from the main producing countries. It satisfies all current legal requirements and specifications for identity and purity.

Specifications established by JECFA are intended to identify the substance that has been subjected to biological testing, mainly for the use of toxicologists and others concerned with standards of identity and purity (FAO, 1982). Should an analytical specification more detailed than any published previously be desired for arbitration purposes in trade or legal disputes, the average values for all

the samples and specimens shown in Table 1 offer the most comprehensive and representative data available. The ranges of values for each analytical parameter in Table 1 reflect the expected seasonal and geographical variations and represent the minimum limits for the extent of the variations that reputable samples of a natural product of such complex structure must be expected to show. The structural data available have been re-interpreted recently (Street and Anderson, 1983) in favour of a more ordered structure than postulated previously. In connection with studies of the immunogenicity of gum arabic, the amino acid compositions of various samples are being investigated and studies made, with the help of new supports for high molecular-weight gel filtration, of the highly proteinaceous fractions of high viscosity that can be isolated (Anderson and Stoddart, 1966).

ACKNOWLEDGMENTS

We thank Rowntree-Mackintosh p.l.c. (York, U.K.), Iranex S.A. (France) and the International Natural Gums Association for Research Ltd. (INGAR) for financial support; officials of MAFF and DHSS (U.K.), F.A.O. (Rome) and W.H.O. (Geneva) for constructive suggestions; Dr. Lawton, Dr. Vassal and (the late) Mr. Vidal-Hall for collection of authentic specimens, and Mr. C.A. Street (Rowntree-Mackintosh p.l.c., U.K.) for arranging the supply of the Test Article and samples 11 and 12.

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RÉSUMÉ

L'échantillon de la gomme arabique commerciale employé comme "L'article d'essai" pour récentes etudes toxicologiques a été caracterisé de manière chimique. Les données comparatives analytiques, présentées pour sept autres échantillons commerciales de la gomme arabique, et pour cinq spécimens de la gomme receuillis d'arbres authentiques d'*Acacia senegal* dans plusieurs pays producteurs, indiquent que l'article d'essai est un équitable, representatif échantillon, établissant la moyenne de la gomme arabique d'origine *Acacia senegal* et conformant en tous rapports aux critères d'identité et de purité établis internationellement.

Phytochemistry, Vol. 23, No. 3, pp. 575–577, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

ACACIA GUM EXUDATES FROM SPECIES OF THE SERIES GUMMIFERAE*

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(*Received* 16 *June* 1983)

Key Word Index—*Acacia*; Gummiferae; gum exudates; chemotaxonomy.

Abstract—An analytical study of the gum exudates from the African species *Acacia ehrenbergiana* (three specimens), *A. xanthophloea* (two specimens), *A. hockii* and *A. sieberana* var. *villosa*, and of the Australian species *A. calcigera*, has been made. There are now 19 species within the series Gummiferae Benth. for which gum parameters are available; of these, only *A. ehrenbergiana* gum displays a slightly negative optical rotation. The data for the three gum specimens from *A. ehrenbergiana* give a further example of the extent to which the gum from different trees of one particular species can vary in composition. The data for *A. sieberana* var. *villosa* gum are compared with the values established previously for subsp. *sieberana*; the differences between varieties of one species are similar in extent to those established for some subspecies. Although *A. xanthophloea*, *A. hockii*, *A. ehrenbergiana*, *A. seyal* and *A. karroo* are regarded as being very closely related botanically, the values for some of their analytical parameters differ considerably and strongly support the view that it is correct to retain them as distinct species.

INTRODUCTION

The analytical data obtained for the gums from *Acacia calcigera*, *A. sieberana* var. *villosa*, *A. xanthophloea* (two specimens), *A. hockii* and *A. ehrenbergina* (three specimens) are shown in Table 1 and compared with the data obtained previously for the gums from *A. sieberana* var. *sieberana* [1], *A. seyal* [2–4] and *A. karroo* [5].

RESULTS AND DISCUSSION

The new data recorded here increase the number of species in Gummiferae Benth. for which analytical data for gum exudates are available [4] to 19. Of these, all except *A. ehrenbergiana* give gums having strongly positive optical rotations, ranging from +28° for *A. hebeclada* [6] to +108° for *A. nilotica* [7]. The data for gum specimens A, B and C from *A. ehrenbergiana* give further examples of the extent of the variations found in the gum nodules exuded by different trees of a particular species, e.g. *A. senegal* (gum arabic) [8], *A. karroo* [5], *A. nilotica* [7], *A. dealbata* [1], *A. laeta* [9], etc.

None of the gum samples studied in this work have high nitrogen contents. The highest nitrogen content recorded so far (9.4%) for an *Acacia* gum is for a Gummiferae species, *A. hebeclada* [6]; several species within the subseries Juliflorae are now known [10] to have nitrogen contents of greater than 7.5%.

It is unusual for species within the Gummiferae to occur in Australia. The gum from *A. calcigera*, a species discovered recently by Dr. Mary Tindale, has been shown (Table 1) to be a typical member of the Gummiferae; it is similar to the gums from *A. drepanolobium*, *A. nilotica* and *A. nubica* [4] in containing less than 1% of rhamnose, a feature also shown by some members of the Juliflorae [10].

The data for the gum from *A. sieberana* var. *villosa* are shown in Table 1 together with the data reported previously for *A. sieberana* var. *sieberana* [1]. Many of their gum parameters are closely similar, e.g. the optical rotations, intrinsic viscosities, molecular weights, and the nitrogen, methoxyl and rhamnose contents, but there are differences in their uronic acid contents and the ratios of galactose to arabinose. The differences between these two *varieties* of *A. sieberana* are comparable with those recorded recently for some subspecies, e.g. for *A. dealbata* [1] and its subspecies *subalpina* [11], *A. mellifera* and its subspecies *detinens* [6], and for three of the subspecies of *A. tortilis* [12, 13]. Although the analytical differences between varieties and between subspecies are not large, they are nevertheless adequate for analytical differentiation for chemotaxonomic purposes.

Acacia xanthophloea, found from Kenya southwards to Swaziland and Zululand, is unique [14] in having either deep yellow or white/purple flowers and these two forms are, in general, clearly differentiated geographically [15]. On this basis, specimens A and B almost surely had white/purple and yellow flowers, respectively (we are grateful to one of the referees for this suggestion). The yellow-flowered specimens have been stated [14] to resemble *A. seyal* and the white/purple flowered specimens to resemble *A. kirkii*. Comparison of the gum data for *A. xanthophloea* specimens A and B and *A. seyal* (Table 1) with that for *A. kirkii* [6] now indicate that although *A. xanthophloea* specimens A and B are closely similar, there does not appear to be any close similarity between the gums from *A. seyal* and yellow-flowered *A. xanthophloea* nor between the gums from *A. kirkii* and white/purple flowered *A. xanthophloea*. In contrast, the gum from *A. seyal* is clearly very similar to that from *A. kirkii*.

Acacia hockii is exceedingly variable and has long been confused with *A. seyal* [14]. Although the two are undoubtedly closely related, Ross [14] believes it to be

*Part 63 of the series "Studies of Uronic Acid Materials".

Table 1. Analytical data for gum polysaccharides from *Acacia* species of the series Gummiferae Benth

	A. calcigera	*A. sieberana* var. *sieberana*	*A. sieberana* var. *villosa*	*A. xanthophloea* A	*A. xanthophloea* B	*A. hockii*	*A. ehrenbergiana* A	*A. ehrenbergiana* B	*A. ehrenbergiana* C	*A. seyal*	*A. karroo*
Moisture (%)	13.1	7.1	6.0	12.7	11.1	4.4	6.0	7.9	12.1	13.4	6.7
Ash (%)*	2.7	1.9	1.5	2.4	5.5	1.3	3.1	3.0	3.5	2.8	3.2
Nitrogen (%)*	0.15	0.35	0.19	0.14	0.39	0.23	0.09	0.12	0.11	0.14	0.15
Hence protein (%) (N × 6.25)*	0.9	2.2	1.2	0.87	2.4	1.4	0.6	0.8	0.7	0.9	0.9
Methoxyl (%)†	0.76	0.74	0.68	2.4	2.0	0.61	0.84	0.56	0.48	0.94	0.47
$[\alpha]_D$ in water (degrees)†	+97	+106	+103	+35	+44	+91	−7	−9	−3	+51	+53
Intrinsic viscosity (ml/g)*	15	12	12	15	24	13	7	8	8	12	17
Molecular weight, MW, $\times 10^5$	26	14	14	9.2	0.4	2.7	2.7	1.1	1.0	8.5	18
Equivalent weight†	1430	2300	1230	1050	1120	1460	1060	810	820	1470	1250
Hence uronic anhydride (%)‡	12	8	14	17	16	12	17	22	22	12	14
Sugar composition after hydrolysis											
4-O-Methylglucuronic acid§	4.5	4.5	4	14.5	12	3.5	5	3.5	3	5.5	2.5
Glucuronic acid	7.5	3.5	10	2.5	4	8.5	12	18.5	19	6.5	11.5
Galactose	34	28	35	54	61	50	56	55	51	38	50
Arabinose	54	60	47	23	16	30	17	13	16	46	28
Rhamnose	< 1	4	4	6	7	8	10	10	11	4	7

*Corrected for moisture content.
†Corrected for moisture and protein content.
‡If all acidity arises from uronic acids.
§If all methoxyl groups located in this acid.

preferable to maintain them as separate species; *A. hockii* differs mainly in not having a powdery bark. As *A. seyal* is also closely related to *A. karroo* [14] and as the absence of a powdery bark suggests [14] that *A. ehrenbergiana* is more closely related to *A. hockii* than to *A. seyal*, comparisons are made of the gum parameters from all of these species in Table 1. The analytical data differ quite extensively, supporting the view [14] that they should all be maintained as separate species. To strengthen these deductions, analytical data for further specimens of the gums from these closely related, variable, species are desirable. Similar evidence from other series in *Acacia*, e.g. Juliflorae [10] and Botryocephalae [11] indicate that the analytical differences between closely related species can be extensive and of potential chemotaxonomic use, as suggested in 1969 [16].

In the past, gums from the series Gummiferae Benth. (e.g. *A. seyal*) have been of minor commercial interest in comparison with those from the series Vulgares Benth.; *A. seyal* gum has long been offered for sale commercially as a commodity distinct from true gum arabic (derived from *A. senegal*, series Vulgares). *Acacia seyal* gum (Talha) behaves differently in confectionery manufacture and in other technological applications and is regarded as being of inferior quality. As toxicological clearance for use in foodstuffs has been accorded by international regulatory committees to gum arabic when defined as "the gummy exudate from *A. senegal* Willd. and the related *Acacia* species" [17, 18], gums from *Acacias* within the series Gummiferae Benth. are excluded and therefore now largely of academic interest only. An implication of the definition adopted is that 'gum arabic', a special term for gum from specified *Acacias*, is no longer synonymous with the general term 'gum acacia'.

EXPERIMENTAL

Origin of gum specimens. Gum from *A. ehrenbergiana* Hayne; specimens A, B and C were collected by Dr. J. Vassal, University of Toulouse, France, near the airport at Nioro du Sahel, Mali, on 16/2/1977. *Acacia xanthophloea* Benth.: gum specimen A was collected in Kenya in August 1976 by Dr. J. O. Kokwaro, Botany Department, University of Nairobi; specimen B, sent by Professor K. D. Gordon-Gray on 19/1/1976, was obtained from a tree cultivated in Pietermaritzburg from seed collected from a tree growing indigenously in Zululand. Gums from *A. hockii* De Wild. and from *A. sieberana* DC. var. *villosa* A. Chev. were collected in April 1975 in Northern Ghana by Dr. M. Jefferies, University of Salford. The identifications of voucher specimens were confirmed by Dr. R. M. Polhill, Royal Botanic Gardens, Kew. Gum from *A. sieberana* DC. var. *sieberana* was collected by Mr. A. G. Seif-el-Din, Gum Research Officer to the Republic of the Sudan, at El Obeid in June 1969. Gum from *A. calcigera* Tindale MS (NSW 108559) was collected by Dr. Mary Tindale (M. Tindale 6075) on 9/7/79, 28.8 km South of Maranboy turn-off on Stuart highway, Northern Territory, Australia.

Preparation of samples for analysis. All the gum specimens dissolved in cold water to give clear solns, which were filtered (muslin, then paper), dialysed for 2 days vs tap water, and freeze-dried.

Analytical methods. The analytical methods used have been described [19].

Acknowledgements—We thank the Science and Engineering Research Council, U.K. for financial support (to MMEB), and the collectors named for providing gum samples for analysis.

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